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THE USE OF VAPOR-HEAT AS A PRACTICAL MEANS OF DISINFECTING SEEDS[1, 2, 3]

P. W. MILLER[4] AND F. P. MCWHORTER[5]

(Accepted for publication September 24, 1947)

Since Pasteur's time numerous studies of the factors that determine the ability of heat to kill microorganisms have been recorded in various bacteriological and physiological texts. These indicate that the higher the water content of the medium in which the organism is contained the lower the temperature and the shorter the exposure required for its death. These observations may have led early phytopathologists, beginning apparently with Jensen in 1887 (2), to utilize hot water to kill pathogens within seeds. Since 1887 specific hot-water treatments for various pathogens and seeds have been developed and recommended. These have not become popular, however, because (a) the treatments require critical time and temperature control, (b) the seeds must be quickly cooled and dried, (c) enormous space is required for drying large amounts of seed, and (d) injury frequently accompanies treatment even when all precautions are observed.

Coincident with the development of the hot-water method of seed disinfection dry heated air was utilized by certain investigators to rid seeds of microorganisms. Critical summaries of these investigations by Atanasoff and Johnson (1) in 1920 and by Lehman (5) in 1925 indicate that temperatures of 95° C. to 100° C. for 1 to 3 days are required for disinfecting seeds with dry heat. Moreover, to avoid injury at the high temperatures, the seeds must be thoroughly dried before beginning the treatment. The long exposures, the possibility of injury, and the contingent fire hazard have discouraged the use of dry heat treatment by commercial seedsmen.

The utility of disinfecting seeds with an intermediate form of heat called "vapor-heat" was investigated by us during 1941 to 1946 as part of the coordinated war-time research program of the American Phytopathological

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[2] The authors are equally responsible for this investigation and manuscript.

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Mrs. Louisa Kanipe, seed analyst, Oregon Agricultural Experiment Station, for valued assistance in determining the viability of many of the seeds tested.

Dr. L. D. Leach for data on the control of *Phoma betae* on sugar beet seeds by vapor-heat.

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Northrup, King and Company for their cordial cooperation in the conduct of certain of the tests reported herein.

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Society. The application of heat in a manner intermediate between heated water and heated dry air is accomplished by introducing steam into a current of air with a mechanical arrangement adjusted to maintain a desired temperature and proportionate moisture content. The term vapor-heat for such steam-air mixtures was apparently first suggested by Hawkins and introduced by Latta (3) in connection with the disinfestation of narcissus bulbs.

A preliminary report of our investigations has already been made (6). This more detailed account is presented to show the practical value and limitations of vapor-heat for seed treatment. The method avoids extreme wetting and drying, thus eliminating many of the objections mentioned above. The principle, if applied in suitable automatic machinery, seems well adapted for large-scale operations by seedsmen.

THE VAPOR-HEAT MACHINE AND METHODS

The equipment used in these tests is essentially the same as devised and described by Latta (3, 4). Steam at 10 to 20 pounds pressure is released through a series of small openings in a U-shaped pipe and mixed or "vaporized" with air in a sheet-metal conditioning box approximately 5 feet long, 3 feet wide, and 3 feet high. From the conditioning box, the vapor passes through a metal conduit into a double-walled, wooden treating room approximately $5 \times 5 \times 4$ feet (Fig. 1). The hot vapor enters the treating room through a square opening in the center of the ceiling and is distributed by a perforated baffle plate suspended about 6 inches below the opening. The perforated plate allows a portion of the mixture to flow downward into the center of the room. The vapor is subsequently withdrawn from the lower part of the room and circulated again through the conditioning box and reintroduced through the ceiling by means of a fan placed in the circulation "stream." The temperature of the mixture is held constant to within $\pm 2°$ F. by Mercoid thermostatic control equipment which operates a steam valve introducing more steam into the mixture as needed to maintain this required temperature.

The use of vapor-heat for seed disinfection being new, a large number of temperature combinations on different seeds and pathogens were tested before a somewhat standardized procedure was attained. Later, we always used low temperature approach periods followed by short higher temperature exposures. Since these temperatures extended from 110° F. to 165° F. and over 800 operations of the vapor machine were involved it is possible to include only a small portion of the records in the accompanying tables. Temperatures and combinations which later experience proved too low have been largely omitted.

The seeds to be treated were placed in small trays, measuring $11 \times 11 \times 3$ cm. with 32-mesh wire screen bottoms and holding approximately 35 gm. of table beet seed and 75 gm. of cabbage seed. The seeds were transferred aseptically to sterile paper sacks after treatment in the vapor-heat machine, and were subsequently plated out on potato-dextrose agar to determine the

viability of the microorganisms present on or within the seeds. At the same time comparable untreated seeds were plated out as controls. Seeds in germination and infection studies were handled in a similar manner.

The viability of seed by laboratory methods was determined as the average germination of two samples of 100 seeds each.

The germination by rate of emergence was determined as two to four replicates of 100 seeds, or seed-balls, each, planted in pasteurized soil. The

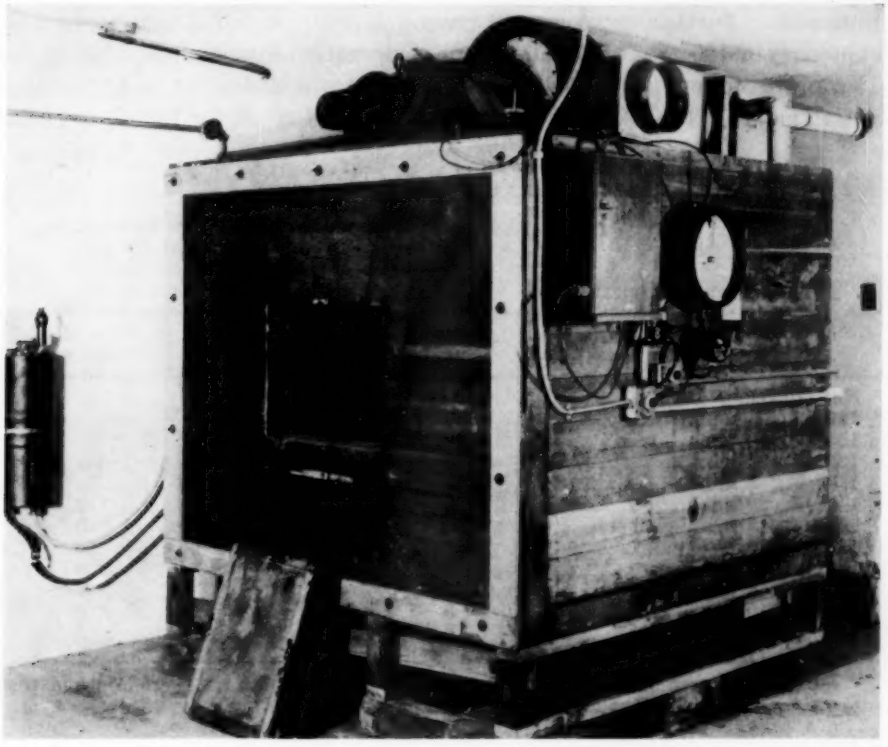

FIG. 1. The vapor-heat machine.

emergence of the seed was recorded as a percentage of the seed sown and the mean emergence period in days was thus determined.

RESULTS

Efficacy of vapor-heat to disinfest seeds contaminated with molds.—Preliminary trials of disinfestation with vapor-heat were made on naturally infested seeds of beet, carrot, cabbage, cauliflower, brussels sprouts, onion, pea, snap bean, radish, red clover, bentgrass, Chewings fescue, and perennial ryegrass. The seeds were randomly infested with species of *Macrosporium*, *Stemphyllium*, *Fusarium*, *Aspergillus*, and *Penicillium*.

Trials included 53 time-temperature variations with or without approach or warm-up periods. These ranged from 117° F. for 30 minutes to 160° F.

for 40 minutes.[6] The results were erratic due to great variation in the fungus flora of different seeds and to the fact that *Penicillium* appeared in cultures representing temperature ranges where control of the other fungi was consistent. We were never certain whether the unpredictable occurrence of *Penicillium* in cultures of treated seeds was attributable to chance recontamination during the transfer of the seeds from the treating room to agar plates, or to resistance of the fungus on the seeds to vapor-heat. Nineteen per cent of 835 seeds plated as controls proved uncontaminated with fungi, thus adding further experimental error. It was concluded that vapor-heat exposures of 150° F. for 30 minutes or combination exposures of 130° F. for 15 minutes followed by 140° F. for 45 minutes will destroy most contaminating molds. The use of an approach period of 130° F. for 15 minutes com-

TABLE 1.—*The effect of vapor-heat on certain fungi*

Vapor-heat treatment	Percentage of seeds inoculated[a] with specified fungi that harbored viable fungus material after vapor-heat treatment for indicated temperatures and exposures		
	Phoma betae	*Botrytis cinerea*	*Fusarium orthoceras*
Not treated	99	98	100
125° F., 5 min.	40	20	.....
125° F., 10 min.	20	0	.....
125° F., 20 min.	0	0	.....
130° F., 5 min.	10	10	.....
130° F., 20 min.	0	0	0
135° F., 5 min.	30	0	0
135° F., 10 min.	20	0	0
135° F., 20 min.	0	0	0
140° F., 5 min.	0	0	0
140° F., 10 min.	0	0	0
140° F., 15 min.	0	0	0

[a] Table beet seed-balls were first autoclaved to kill the natural microflora and then soaked in spore suspensions of the respective fungi.

bined with a short treatment of 140° F. to 145° F. proved consistently better than single treatments of 150° F. for proportional periods. There was some indication that smooth seeds, such as cabbage, were more easily disinfected than such rough seeds as beet "seed-balls."

Ability of vapor-heat to kill specific fungi on and in beet "seed-balls."—The vapor-heat "death points" for *Phoma betae* (Oud.) Frank, *Botrytis cinerea* Pers., and *Fusarium orthoceras* Ap. et Wr. were obtained by first soaking autoclaved beet seed-balls in suspensions of the spores of the respective fungi and allowing the fungi to grow into the coats of the fruits.

The data given in table 1 show that *Fusarium orthoceras* was especially susceptible to vapor-heat and that exposure to a temperature of 140° F. for 20 minutes should kill any of the three fungi when in a vegetative condition.

[6] These data are too voluminous to warrant tabulation and are therefore merely summarized in this paragraph. Also, the many variables kept this preliminary study from being precise.

Vapor-heat "death points" of sclerotia of Sclerotinia.—The very short exposures and relative low temperatures required to inactivate sclerotia of *Sclerotinia minor* Jagger and *Sclerotinia sclerotiorum* (Lib.) De Bary from cabbage are of special interest. Table 2 shows that sclerotia of *S. minor* were killed by vapor-heat treatment at only 130° F. for 15 minutes. This is at least 15 degrees F. and 45 minutes' exposure below the analogous thermal death-point of cabbage seed (Table 4). Platings of sclerotia that were cut open to eliminate error from delayed germination showed that all sclerotia of both species were killed by treatments of 135° F. for 30 minutes. Since many sclerotial "pellets" sometimes remain among cabbage seed after mechanical cleaning, vapor-heat may prove advantageous for disinfesting cabbage seed.

Specific control of seed-borne diseases.—The results of limited studies of controlling specific seed-borne diseases by vapor-heat indicate that this

TABLE 2.—*The effect of vapor-heat on the sclerotia of Sclerotinia from cabbage.*

Organism	Vapor-heat treatment	Number of sclerotia plated	Percentage of sclerotia viable after treatment
Sclerotinia sclerotiorum	Not treated	19	100
Do	130° F., 15 min.	16	31
Do	130° F., 30 min.	6	33
Do	130° F., 45 min.	10	10
Do	130° F., 60 min.	11	27
Do	135° F., 15 min.	6	0
Do	135° F., 30 min.	6	0
Do	135° F., 45 min.	6	0
Sclerotinia minor	Not treated	7	100
Do	130° F., 15 min.	6	0
Do	130° F., 30 min.	6	0
Do	130° F., 45 min.	6	0

method of thermal therapy is practical for certain diseases. However, disinfection of large seeds such as *Phaseolus vulgaris* L., variety White Kidney, infected with *Colletotrichum lindemuthianum* (S. & M.) Bri. & Cav., which penetrates deeply into the seeds is difficult and may be impractical.

The control of *Phoma betae* in naturally infected sugar beet seed-balls by vapor-heat has been demonstrated as recorded in table 7. Moreover, preliminary tests indicate that the vapor-heat treatment of table beet fruits naturally infected with *Phoma betae* may prevent the crown rot disease of table beet roots. This was indicated by tests carried on in 1944–1945 in cooperation with Northrup, King and Company of Minneapolis, Minnesota. In February, 1944, we vapor-heat treated some naturally infected table beet seeds supplied by this company. These seeds were planted by representatives of the company in their trial grounds in Minneapolis along with non-treated seeds from the same source. The roots from both lots were harvested in October, 1944, and stored in a cellar over winter. The treated lot was kept separate from the non-treated lot. When the roots went into storage there was no detectable difference in the two lots. The roots were subse-

quently removed from storage about the middle of April, 1945, and the incidence of "rotted" roots in each lot determined. The untreated roots had about 40 per cent crown-rot while the roots produced from the seeds treated by vapor-heat were practically free from crown-rot, only 9 roots being affected in some seven bags of roots stored.

As was discussed above, the sclerotia of both *Sclerotinia minor* and *S. sclerotiorum* mixed among cabbage seed are easily killed by vapor-heat treatments that are considerably below the maximum treatment that could be used without causing injury to the seed. Treatment of infested seed by vapor-heat is a practical method of preventing this disease from being seed-borne.

TABLE 3.—*The effect of vapor-heat on three bacterial plant pathogens of the genus Xanthomonas*

Host	Bacterial pathogen	Vapor-heat treatment	Number of seeds[a] cultured	Percentage of seeds containing viable bacteria after treatment
Cabbage	*X. campestre*	Not treated	60	58
Do	Do	145° F., 30 min.	20	15
Do	Do	145° F., 60 min.	30	0
Do	Do	145° F., 90 min.	30	0
Do	Do	150° F., 30 min.	10	33
Do	Do	150° F., 60 min.	10	33
Do	Do	150° F., 90 min.	10	0
Snap beans	*X. phaseoli*	Not treated	101	98
Do	Do	147° F., 30 min.	12	58
Do	Do	152° F., 30 min.	19	58
Do	Do	152° F., 60 min.	12	25
Do	Do	157° F., 30 min.	12	33
Do	Do	157° F., 75 min.	12	33
Do	Do	157° F., 90 min.	12	0
Do	Do	162° F., 30 min.	12	17
Do	Do	162° F., 45 min.	12	0
Carrot	*X. carotae*	Not treated	65	97
Do	Do	142° F., 30 min.	14	14
Do	Do	147° F., 30 min.	14	7
Do	Do	152° F., 30 min.	33	15
Do	Do	157° F., 30 min.	17	18

[a] Seeds autoclaved, then soaked in aqueous suspensions of the organism in question and subsequently air-dried under aseptic conditions.

Special cases for application of thermal disinfection by means of vapor-heat.—The vapor-heat method possesses many other possible control potentialities that invite investigation. For example, preliminary tests indicate that it may be used effectively to disinfect plant parts other than seeds, such as onion bulbs, gladioli corms, etc. Many other uses may be found where this easily controlled method of heat treatment may be applied without introducing a difficult problem of drying.

Vapor-heat impractical for control of bacterial diseases.—Bacteria are much more difficult to kill by vapor-heat than are fungi. It has proved impossible to disinfest seeds contaminated with spore-forming bacteria at

temperature ranges herein reported. While non spore-forming bacterial plant pathogens are eventually killed, the temperatures required are too high for most seeds to endure without serious injury. Table 3 shows the control potential of vapor-heat on *Xanthomonas campestre* (Pammel) Dowson, *X. phaseoli* (E. F. Smith) Dowson, and *X. carotae* on cabbage, snap bean, and carrot seeds, respectively. It is apparent from these data that the vapor-heat method alone is impractical for controlling seed-borne bacteria. This observation led to preliminary trials of vapor-heat followed by application of chemical disinfectants. A test by plating technique to compare the relative efficiency of vapor-heat and copper oxide for disinfesting beet fruits showed that a combination of the two attained complete disinfestion. The copper alone did not prevent growth of fungi; the heat alone did not prevent growth of bacteria. Combinations of vapor-heat and chemical seed treatment may lead to better control of seed-borne and soil-borne pathogens.

Tolerance of seeds to vapor-heat.—Seeds of Kentucky Wonder bean, brussels sprouts, Marion Market cabbage, Chantenay carrot, Early Sweet pea, sugar beet, Detroit Dark Red table beet, red clover, bentgrass, Chewings fescue, and perennial ryegrass were tested for tolerance to vapor-heat treatments. The tolerance as recorded in table 4 was determined by (a) standard laboratory seed analysis methods, and (b) the rate of emergence in pasteurized soil. These data show that while the tolerance to vapor-heat of individual kinds of seeds varies considerably, many kinds withstand significantly higher temperatures than are required to kill associated fungi. Thus, table beet seed-balls can evidently endure vapor-heat treatment up to 145° F. for 30 minutes with little, if any, effect on the germination or rate of emergence. This treatment is some 10 degrees F. higher than is required to kill most of the associated fungi.

The wide difference in the vapor-heat tolerance of some of the seeds of closely related plants is noteworthy. For example, a vapor-heat treatment of 145° F. for 45 minutes which has little, if any, effect on the germination of cabbage, may seriously injure seeds of cauliflower and brussels sprouts. Similar variations among varieties or lots of the same species may be anticipated (Table 5).

In selecting the vapor-heat temperatures and exposure periods for any particular seed problem it is essential to employ those that retard germination the least and yet are efficacious. If germination is retarded by the treatment deemed necessary to effect disinfection, the seeds should subsequently be treated with a suitable protectant to avoid damping-off during the lengthened pre-emergence period.

The effect of age of seed on susceptibility to injury by vapor-heat.—It may be presumed that any circumstance that naturally weakens the viability of seeds would lend to proportionately greater injury from vapor-heat treatment. Aging of seed is a common circumstance that militates against good germination. One test with cauliflower seed representing 0, 1, 2, and 3 years of aging respectively, reported in table 5, shows that injury to cauli-

TABLE 4.—*The effect of vapor heat on the germination of certain seeds*

Seed and series number[a]	Vapor-heat treatment		Germination in laboratory[b]	Germination in greenhouse	
	Approach period	Standard period		Seedlings emerged[c]	Mean emergence period
			Per cent	*Per cent*	*Days*
Table beet					
1, a	.....	Not treated	77	94	.....
1, b	.....	145° F., 15 min.	77	.....	.....
1, c	.....	145° F., 30 min.	79	.....	.....
1, d	.....	145° F., 45 min.	74	72	.....
1, e	.....	145° F., 60 min.	77	53	.....
1, f	.....	145° F., 90 min.	68	52	.....
1, g	.....	150° F., 20 min.	89	138	.....
1, h	.....	150° F., 30 min.	78	.....	.....
1, i	.....	150° F., 45 min.	70	.....	.....
1, j	.....	150° F., 60 min.	83	80	.....
1, k	.....	150° F., 90 min.	72	.....	.....
1, l	.....	155° F., 15 min.	87	.....	.....
1, m	.....	155° F., 30 min.	72	.....	.....
1, n	.....	155° F., 45 min.	72	.....	.....
1, o	.....	155° F., 60 min.	78	.....	.....
1, p	.....	160° F., 20 min.	76	.....	.....
1, q	.....	160° F., 40 min.	46	2	.....
1, r	.....	160° F., 60 min.	9	0	.....
1, s	.....	160° F., 80 min.	1	0	.....
1, t	.....	165° F., 20 min.	70	.....	.....
1, u	.....	165° F., 40 min.	38	.....	.....
1, v	.....	165° F., 60 min.	11	.....	.....
2, a*	.....	Not treated	.....	91	.....
2, b*	130° F., 15 min.	140° F., 30 min.	.....	89	.....
3, a	.....	Not treated	.....	168	5.5
3, b	130° F., 15 min.	140° F., 45 min.	.....	97	6.5
4, a	.....	Not treated	.....	130	8
4, b	135° F., 15 min.	140° F., 60 min.	.....	134	8
5, a	.....	Not treated	.....	162	5.5
5, b	130° F., 10 min.	145° F., 30 min.	.....	157	5.5
6, a	.....	Not treated	.....	79	.....
6, b	135° F., 15 min.	145° F., 45 min.	.....	85	.....
7, a	.....	Not treated	.....	256	4
7, b	135° F., 15 min.	146° F., 30 min.	.....	196	5.5
8, a	.....	Not treated	.....	180	5
8, b	135° F., 20 min.	146° F., 45 min.	.....	147	6.5
Sugar beet					
9, a	.....	Not treated	89	179	.....
9, b	130° F., 15 min.	140° F., 30 min.	90	185	.....
10, a	.....	Not treated	.....	226	5.5
10, b	130° F., 15 min.	140° F., 35 min.	.....	237	5
Carrot					
11, a	.....	Not treated	.....	45	6.5
11, b	135° F., 15 min.	146° F., 30 min.	.....	33	7.5
12, a	.....	Not treated	.....	46	7.5
12, b	135° F., 20 min.	146° F., 45 min.	.....	17	9
13, a	.....	Not treated	.....	75	13
13, b	.....	150° F., 45 min.	.....	40	17.5
14, a	.....	Not treated	.....	45	6.5
14, b	135° F., 20 min.	146° F., 30 min.	.....	33	7.5

TABLE 4.—(*Continued*)

Seed and series number[a]	Vapor-heat treatment		Germination in laboratory[b]	Germination in greenhouse	
	Approach period	Standard period		Seedlings emerged[c]	Mean emergence period
			Per cent	*Per cent*	*Days*
15, a	.....	Not treated	.....	46	7.5
15, b	135° F., 20 min.	146° F., 45 min.	.....	17	9
16, a	.....	Not treated	.....	75	13
16, b	.....	150° F., 45 min.	.....	40	17.5
Cabbage					
17, a	.....	Not treated	95	84	.....
17, b	.....	145° F., 30 min.	96.5	.....	.....
17, c	.....	145° F., 45 min.	97	.....	.....
17, d	.....	145° F., 60 min.	95	.....	.....
17, e	.....	150° F., 30 min.	93	.....	.....
17, f	.....	150° F., 60 min.	88.5	.....	.....
17, g	.....	150° F., 90 min.	66.5	.....	.....
17, h	135° F., 20 min.	145° F., 45 min.	82.7	88	.....
17, i	130° F., 20 min.	150° F., 30 min.	89	52	.....
17, j	135° F., 20 min.	150° F., 60 min.	70.5	40	.....
17, k	130° F., 20 min.	150° F., 90 min.	23.5	6	.....
17, l	130° F., 20 min.	150° F., 120 min.	5	8	.....
Brussels sprouts					
18, a	.....	Not treated	.....	76	6.0
18, b	132° F., 15 min.	137° F., 20 min.	.....	79	6.0
19, a	.....	Not treated	.....	89	6.5
19, b	137° F., 15 min.	147° F., 30 min.	.....	28	11
Cauliflower					
20, a	.....	Not treated	93	.....	.....
20, b	.....	135° F., 30 min.	91	.....	.....
20, c	.....	140° F., 30 min.	91	.....	.....
20, d	.....	148° F., 30 min.	32.4	.....	.....
21, a	.....	Not treated	71	.....	.....
21, b	135° F., 20 min.	145° F., 45 min.	0	.....	.....
Peas					
22, a	.....	Not treated	94.5	.....	.....
22, b	.....	160° F., 20 min.	90	.....	.....
22, c	.....	160° F., 40 min.	76.5	.....	.....
22, d	.....	160° F., 60 min.	66	.....	.....
22, e	.....	160° F., 80 min.	55.5	.....	.....
Beans					
23, a	.....	Not treated	.....	95	6
23, b	.....	146° F., 60 min.	.....	92	6.5
24, a	.....	Not treated	.....	97	9.5
24, b	.....	150° F., 45 min.	.....	66	11

[a] An asterisk indicates that the seeds in this series were dipped in a spore suspension of *Phoma betae* before treatment.

[b] Average germination, in per cent, of 2 samples of 100 seeds, or seed-balls, each as determined by standard laboratory seed analysis methods.

[c] Seedlings emerged in percentage of seeds, or seed-balls, sown.

flower seed increases with age and that the process is more suitable for current-season seed. The germination of the fresh seeds treated at the higher temperature level was from 3 to 28 times that of the 3-year-old seed. The strain Snowball "A" was consistently more resistant to injury than Snowball "X."

The proper interval between harvesting seeds and vapor-heat treatment has not been investigated. This relationship should be determined for various kinds of seeds and the diseases associated with them.

The relation of depth of seeds to treating efficiency.—We had hoped that vapor-heat would penetrate sufficiently to permit treating of seeds in sacks. That treatment in sacks is impractical was proved by determining how far

TABLE 5.—*The effect of vapor-heat on the germination of cauliflower seed of different ages, 1946*

Year seed grown	Vapor-heat treatment	Average germination of two strains of seed as determined by standard laboratory methods	
		Snowball "A"	Snowball "X"
		Per cent	*Per cent*
1942	Not treated	90	97
Do	135° F., 30 min.	91	95
Do	140° F., 30 min.	91	95
Do	148° F., 30 min.	20	2
1943	Not treated	93	88
Do	135° F., 30 min.	87	90
Do	140° F., 30 min.	92	91
Do	148° F., 30 min.	54	35
1944	Not treated	95	91
Do	135° F., 30 min.	91	95
Do	140° F., 30 min.	94	92
Do	148° F., 30 min.	46	0
1945	Not treated	94	99
Do	135° F., 30 min.	97	94
Do	140° F., 30 min.	92	95
Do	148° F., 30 min.	73	56

within a mass of beet fruits complete disinfestation was obtained. The beet seed-balls used, as reported in table 6, were contaminated randomly with species of *Stemphyllium, Macrosporium,* and *Penicillium.* Decontamination is significantly reduced at distances of more than one inch within the seed mass even in the case of a favorable subject such as beet seed-balls. The self-insulation against heat provided by small seeds in masses would obviously be more pronounced. This relatively poor penetration must be considered in planning commercial applications of vapor-heat and is discussed later in this paper.

The absorption of water by seeds during vapor-heat treatment.—Seeds treated with hot water absorb from ten to twenty times as much water as those treated by vapor-heat (Table 8). This is a significant advantage of vapor-heat over the hot water method. While all vapor-heat-treated seeds

TABLE 6.—*The relation of the depth of table beet seed-balls in containers to decontamination by vapor-heat*

Depth of seed in trays in millimeters	Vapor-heat treatment		Number of seed-balls cultured	Percentage of seed-balls harboring viable fungi after treatment
	Approach period	Standard period		
.....	.....	Not treated	352	97
2 to 5	135° F., 15 min.	140° F., 30 min.	30	0
25	130° F., 15 min.	140° F., 30 min.	34	44
75	130° F., 10 min.	140° F., 30 min.	15	93
2 to 5	135° F., 15 min.	145° F., 30 min.	30	0
25	135° F., 15 min.	145° F., 30 min.	42	28
75	135° F., 15 min.	146° F., 30 min.	30	70
2 to 5	135° F., 15 min.	145° F., 45 min.	10	0
25	135° F., 20 min.	145° F., 45 min.	50	4
50	135° F., 20 min.	146° F., 45 min.	15	40
95	135° F., 20 min.	146° F., 45 min.	14	64
2 to 5	135° F., 20 min.	150° F., 30 min.	8	0
25	135° F., 20 min.	150° F., 30 min.	20	0
2 to 5	135° F., 20 min.	150° F., 60 min.	8	0
25	135° F., 20 min.	150° F., 60 min.	30	0
2 to 3	135° F., 20 min.	150° F., 90 min.	8	0
25	135° F., 20 min.	150° F., 90 min.	10	0
2 to 3	.....	145° F., 15 min.	8	50
25	.....	145° F., 15 min.	10	30
2 to 3	.....	145° F., 30 min.	9	0
25	.....	145° F., 30 min.	20	20
2 to 3	.....	150° F., 120 min.	8	0
20	.....	150° F., 120 min.	20	10

appear only slightly dampened, those with porous coats, such as beet seed-balls, absorb more moisture during treatment than seeds with hard "flinty" coats, such as cabbage. Most treated seeds were almost dry enough to "pour" when removed from the machine. The moisture content can be

TABLE 7.—*The control of Phoma betae in naturally infected sugar beet seed-balls by vapor-heat treatment*[a]

Series	Treatment	Emergence per 100 seed-balls	Seedlings "damped-off" after emergence	Seedlings with root lesions[b]	Total seedlings infected
			Per cent	*Per cent*	*Per cent*
1, a	None	179	50.6	30.1	80.7
1, b	Vapor-heated (130° F. for 10 min. + 145° F. for 30 min.)	185	2.7	3.9	6.6
2, a	None	142	53.5	28.9	82.4
2, b	Vapor-heated (130° F. for 10 min. + 145° F. for 30 min.)	189	1.8	0.6	2.4

[a] The seeds were vapor-heat treated by us and forwarded to Dr. L. D. Leach who carried on the emergence studies.

[b] All seedlings with root and hypocotyl infections were cultured by Dr. Leach to verify *Phoma betae* as the causal organism.

TABLE 8.—*The percentage of moisture absorbed by certain seeds during vapor-heat treatment as compared with that absorbed during hot-water treatment*

Seed	Treatment	Exposure temperature in degrees Fahrenheit and time in minutes	Average[a] percentage of moisture absorbed during treatment expressed as percentage of original dry weight of seed
Table beet	Hot-water	134° F. for 10 minutes	98.8
Do	Vapor-heat	130° F. for 10 minutes + 145° F. for 30 minutes	4.1
Do	Vapor-heat	135° F. for 15 minutes + 140° F. for 60 minutes	5.9
Cabbage	Hot-water	122° F. for 15 minutes	27.0
Do	Vapor-heat	130° F. for 10 minutes + 145° F. for 30 minutes	2.8

[a] Average of 4 replicates.

lowered to the initial dryness by introducing hot air into the chamber before removing the seeds. Proper mechanical equipment for vapor-heat treatment of seeds would dry the seeds automatically before they are stored.

DISCUSSION

The foregoing data indicate that the vapor-heat principle may be used to disinfect and disinfest seeds without serious injury. In many cases reported above, effective treatment was accomplished at levels far below the point of injury to the seeds. Precise temperature control is not a critical factor and the treatment periods are not excessively long. It is likely that vapor-heat treatments can be designed that will effectively replace hot-water treatment of seeds in those cases where thermal disinfection has proved necessary.

The data presented show that because of irregular and inadequate penetration seeds could not be treated in sacks. Practical application therefore would require the construction of special vapor-heat machinery not unlike some of the equipment already used by canners. The seeds could be carried along on slowly moving belts through tunnel-like channels wherein the vapor-heat would be applied. In the last section of the tunnel they could be exposed to warm, dry air so that they would emerge dry enough for final storage. The amount of moisture left on the seed could be so adjusted that a suitable post-emergence protectant could be more effectively applied before they are placed in containers for marketing.

SUMMARY

A method for disinfecting seeds by vapor-heat is described.

Tabulated data presented indicate that certain fungi are killed at relatively low vapor-heat temperatures for short exposures. However, most bacteria are not killed by vapor-heat treatments that are safe for seeds, and the control of bacterial seed-borne diseases by vapor-heat alone seems impractical.

Certain kinds of seeds will apparently endure considerably higher vapor-heat temperatures than are required to kill all associated fungi. Thus, table beet seed-balls will evidently tolerate a vapor-heat temperature of around 145° F. for as long as 30 minutes without significant reduction or retardation of germination. *Phoma betae,* an associated pathogen, is killed at 135° F. for 20 minutes, which is about 10 degrees F. lower than the phyto-lethal temperature.

The practical control of *Phoma betae* on and within sugar beet and table beet seed-balls is indicated by these investigations. Preliminary tests also indicate that the method will kill sclerotia of *Sclerotinia* occurring as a contaminant of cabbage seed.

The important advantages of vapor-heat treatment are (a) temperature control is not critical as for hot water treatment, (b) treatment periods are short in comparison with dry heat, and (c) the difficulty of drying seed which limits the use of hot water is eliminated by proper vapor-heat procedures.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING, AGRICULTURAL RESEARCH ADMINISTRATION,
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EFFECT OF SEVERAL SEED PROTECTANTS ON EMERGENCE AND STAND OF OKRA[1]

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(Accepted for publication October 14, 1947)

During the summer months in the coastal area of South Carolina, okra is the most dependable vegetable for the home garden. A considerable acreage is also grown for canning near Charleston. A satisfactory stand of okra plants is often difficult to obtain. Since information regarding okra seed treatment was lacking, experiments with seed protectants[3] were conducted. Results indicate that the use of such materials aids greatly in securing satisfactory stands. A brief summary of the results with the most promising materials has been presented in South Carolina Agricultural Experiment Station Bulletin 361.

One experiment in 1942, two in 1943, and one in 1944 were made in sandy loam soil. Each test was made in a different field. A randomized block design with 5 or 6 replicates of each treatment was used for each test. On each plot consisting of a single row 10 or 12 feet in length, 100 seeds were planted. Clemson Spineless okra seed was used exclusively in all experiments except No. II in which several varieties were included.

Experiment I.—Seed was treated on March 10 and kept in paper bags until planted on March 31, 1942. This was about three weeks earlier than the normal seeding date. The plants that emerged were counted on April 23 and the surviving plants on April 29 (Table 1). The germination of the seed used was low, which seems to be normal for many lots of okra seed.

A second test, similar in design and materials to the first one, was seeded on August 11, 1942. Plants were emerging on August 17. Stand data (Table 1), taken on August 26, indicate the value of the various treatments.

Experiment II.—Spergon and New Improved Ceresan, which appeared to be beneficial in 1942, were tested on seed of six varieties of okra in 1943. The seed was treated on March 4 and kept in closed containers for 24 hours. Each plot, a single 12-foot row, was seeded on March 24.

The average number of plants, for each variety and treatment, emerged by April 12 is shown in table 2. Both Spergon and New Improved Ceresan caused highly significant increases in stand from certain seed lots in com-

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[3] Of the materials used, New Improved Ceresan (5 per cent ethyl mercury phosphate), 2 per cent Ceresan (2 per cent ethyl mercury chloride), Semesan (30 per cent hydroxymercurichlorophenol), Arasan (50 per cent tetramethylthiuram-disulfide), Fermate (70 per cent ferric dimethyldithiocarbamate), were furnished by E. I. DuPont de Nemours & Co., Spergon (tetrachloro-para-benzoquinone), Spergonex (o-benzoquinone dionium peroxide), and Phygon (2,3-dichloro-1,4-naphthoquinone) by the U. S. Rubber Co. (Naugatuck Division); Cuprocide (96.5 per cent red cuprous oxide) by Rohm and Haas Co.; Tribasic copper sulfate (52 per cent metallic copper) by the Tennesse Copper Company; Seed Disinfectant No. 5 (tetrachloroquinone), and Seed Disinfectant No. 6 (2,4,5-trichlorophenol) by the Dow Chemical Co.

TABLE 1.—*Relation of treatment of okra seed to stand, 1942*

Treatment[a]	Average number of plants per plot[b]		
	Test 1		Test 2
	Emerged	Survived	Emerged
	April 23	April 29	August 26
None (Control)	8.3	6.3	55.8
New Improved Ceresan, 0.5 per cent	32.7**	19.2**	73.8**
Spergon, 1 per cent	27.2**	24.2**	71.7**
Spergon, 0.5 per cent	.....	.....	69.7**
Arasan, 0.5 per cent	29.8**	22.8**	64.5*
Arasan, 0.2 per cent	24.2**	16.2**	.....
2 per cent Ceresan, 0.5 per cent	24.3**	16.5**	.....
Tribasic Copper Sulfate, 2 per cent	22.0**	13.5*	.....
Cuprocide, 2 per cent	15.5*	13.2*	.....
Semesan, 0.5 per cent	20.0**	12.7*	.....
Zinc oxide, 2 per cent	16.0*	10.8	.....
Spergonex, 2 per cent	8.0	6.0	.....
Soaked in water for 20 hours	.....	.....	32.3
Difference required for significance			
at 5 per cent level	6.7	5.8	8.5
at 1 per cent level	8.7	8.2	11.4

[a] Rate of application is expressed as percentage of weight of seed.
[b] Figures marked with one and two asterisks are significantly higher than those for the untreated seed at the 5 per cent and 1 per cent levels, respectively.

parison with the untreated controls. At the dosages used, there were no significant differences between the means for the two materials.

Experiment III.—A dosage series was tried in 1943 in order to learn

TABLE 2.—*Relation of treatment of seed of okra varieties to stand, 1943*

Variety	Average number of plants emerged on April 12[a]		
	Untreated	New Improved Ceresan, 0.5 per cent	Spergon 1 per cent
1. Clemson Spineless	46.6	67.2**	68.4**
2. Carrother's Green Velvet	18.0	13.6	15.6
3. Louisiana White Velvet	62.8	68.6	76.8**
4. Asgrow White Velvet	34.8	41.2	41.6
5. Dwarf Green	49.4	55.0	52.2
6. Perkin's Mammoth	49.2	50.8	51.2
Difference required for significance			
at 5 per cent level	8.8	8.8	8.8
at 1 per cent level	11.6	11.6	11.6
Mean for treatments	43.5	49.4**	51.0**
Difference between treatment means required for significance			
at 5 per cent level			3.58
at 1 per cent level			4.74

[a] Figures marked with two asterisks are significantly higher at the 1 per cent level than those for the untreated seed.

TABLE 3.—*Relation of the treatment of Clemson Spineless okra seed to stand, 1943*

Treatment	Average number of plants emerged on April 29[a]			
	Dosage, percentage by weight			Mean
	0.25	0.5	1	
New Improved Ceresan	82.6	76.6	76.0	78.3**
Spergon	74.4	74.2	75.8	74.8*
Arasan	76.6	71.4	74.0	74.0*
Fermate	75.2	69.6	76.2	73.7*
2 per cent Ceresan	70.4	74.8	72.2	72.5
Semesan	68.4	65.8	66.6	66.9
None (Control)	.....	.....	.....	68.6
Soaked in water for 3 hours	.....	.....	.....	58.8
Mean for dosage[b]	74.6	72.1	73.5	.....
Difference required for significance at 5 per cent level				4.98
at 1 per cent level				6.63

[a] Figures marked with one and two asterisks are significantly higher than those for the untreated seed at the 5 per cent and 1 per cent levels, respectively.

[b] No significant difference between means for dosages.

TABLE 4.—*Relation of treatment of Clemson Spineless okra seed to seedling emergence, 1944*

Treatment		Average number of plants per plot[a]
Material	Dosage (Percentage by weight)	Emerged May 2
None (Control)	.....	38.6
Spergon	4	40.8
Do	2	58.6*
Do	1	57.6*
Do	0.25	57.2*
Do	0.062	44.2
Arasan	2	51.8
Do	1	54.2
Do	0.25	56.0*
Do	0.062	52.2
2 per cent Ceresan	1	46.4
Do	0.25	52.6
Do	0.062	53.6
Do	0.016	52.8
New Improved Ceresan	1	49.6
Do	0.25	52.2
Do	0.062	60.0*
Do	0.016	37.4
Fermate	2	46.6
Do	1	52.0
Do	0.25	48.8
Do	0.062	48.8
Phygon	0.25	48.2
Seed Disinfectant No. 5	0.25	45.4
Do No. 6	0.25	37.6
Difference required for significance at 5 per cent level		17.4
at 1 per cent level		23.0

[a] Figures marked with an asterisk are significantly higher at the 5 per cent level than those for the untreated seed.

the relative effectiveness of three rates of application of several materials in increasing the stands. Seed was treated on March 23, kept in closed glass containers for 24 hours, and planted on April 9. Treatment with New Improved Ceresan at 0.25 per cent dosage resulted in the best stand (Table 3). The differences between the means for dosages were not significant.

Experiment IV.—Seed of the Clemson Spineless variety and the required amount of the dry chemical for each treatment listed in table 4 were mixed together in an electric rotary mixer at 30 to 50 r.p.m. for 4 minutes on February 9, 1944. Soil moisture was optimum at planting on April 7 but was unusually high for several weeks that followed. Counts of the plants emerged by May 2 are given in table 4.

SUMMARY

Treatment of okra seed with either New Improved Ceresan or Spergon resulted in a significant increase in stand in each of five tests. In the tests made, treatment with New Improved Ceresan at the rate of 4 ounces, Spergon at 16 ounces, or Arasan at 4 ounces per 100 pounds of seed was beneficial, whereas treatment with Fermate, 2 per cent Ceresan, or Semesan was beneficial to a lesser degree. In preliminary tests tribasic copper sulfate, Cuprocide, zinc oxide, Phygon, Seed Disinfectant No. 5, Seed Disinfectant No. 6 and Spergonex were not very promising as protectants for okra seed. Soaking the seed in water for several hours prior to seeding resulted in a decrease in the stand.

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A COMPARISON OF HOME-MADE BORDEAUX MIXTURE WITH OTHER FUNGICIDES FOR CONTROL OF SCAB ON THE SCHLEY AND MOORE VARIETIES OF PECAN

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(Accepted for publication October 4, 1947)

During the past 12 years, 1935–1946, the writer has used a number of fungicides (Table 1) in comparison with home-made Bordeaux mixture[2] in order to control scab (*Cladosporium effusum* (Wint.) Demaree), a disease that is one of the limiting factors in pecan production.

The tests were made on the Schley variety of pecan in south Georgia, as well as the Moore variety in north Florida. The Schley is highly susceptible to the scab disease in the locality where the tests were conducted. While the Moore is classed as resistant to scab in most localities, it is susceptible in north Florida.

Each test plot contained a minimum of 9 trees, with an average of 12. A high-power spray machine that maintained a pressure of 400 to 600 pounds with the spray gun open was used in making the 4 spray applications on all test plots.

The purpose of these tests was to find a fungicide that would give commercial control of scab or compare favorably with home-made Bordeaux mixture, an effective and economical fungicide but one that apparently has a tendency to increase the infestation of the black pecan aphid, *Melanocallis caryaefoliae* (Davis).

Table 1 indicates that of the various fungicides used in spraying the Schley variety to control scab during the period 1935–1946, inclusive, Bordeaux mixture gave a greater increase in yield over the unsprayed checks than other fungicides that were tested. In addition, with the exception of 1943 when the check trees were accidentally sprayed one time, the trees sprayed with Bordeaux mixture always gave an increase in yield over the unsprayed checks. Likewise, in 1943 when the check trees gave a higher yield of nuts than the ones that were sprayed 4 times, the trees sprayed with Bordeaux mixture produced more nuts than those trees sprayed with Fermate, or Fermate plus lime.

The set of nuts was greater on the check trees in 1943 than it was on the sprayed ones. Under favorable weather conditions, and with good nutrition of the check trees, *i.e.*, very little premature defoliation from the scab disease and no second growth the preceding year, the check trees occasionally bloom more heavily than the sprayed ones, especially in the "off-

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[2] Prepared by dissolving 6 pounds of snow form copper sulfate and adding 2 pounds of hydrated lime, 400-mesh, analyzing at least 98 per cent calcium hydroxide, in each 100 gallons of water.

TABLE 1.—*Fungicides used in past 12 years for control of pecan scab on the Schley and Moore varieties at Albany and Fort Valley, Georgia, and Monticello, Florida*

Varieties and treatment	Amount of fungicide in 100 gal. of water	Year treated	Av. yield of nuts per tree	Increase or decrease in yield of sprayed trees over unsprayed checks
			lb.	*lb.*
Schley Variety				
Bordeaux mixture	6–2 (6 copper-2 lime)	1935	30	15
Zeolite (sodium silicate)	3 lb.	do	17	2
Coposil (copper silicates and zinc)	5 lb.	do	11	– 4
Copper phosphate ($Cu_3(PO_4)_2 \cdot 3H_2O$)	4 lb.	do	15	.....
Check	.....	do	15	.....
Bordeaux mixture	6–2	1936	55	41
Tribasic copper sulfate (53 per cent metallic copper-basic copper sulfate)	6 lb.	do	19	5
Wettable sulfur (S)	6 lb.	do	15	1
Check	.....	do	14	.....
Bordeaux mixture	6–2	1937	73	66
Cupro-K (copper oxychlorides)	6 lb.	do	14	7
Check	.....	do	7	.....
Bordeaux mixture	6–2	1938	37	22
Tenn. Copper 53 per cent (basic copper sulfate)	3 lb.	do	20	5
Tenn. Copper 26 per cent (basic copper sulfate)	3 lb.	do	18	3
Copper oxalate ($CuC_2O_4 \cdot \frac{1}{2}H_2O$)	4 lb.	do	14	– 1
Check	.....	do	15	.....
Bordeaux mixture	6–2	1939	62	58
Cuprocide (cuprous oxide)	3 lb.	do	8	4
Check	.....	do	4	.....
Bordeaux mixture	6–2	1940	34	7
Yellow copper oxide (yellow cuprous oxide)	1½ lb.	do	25	– 2
Cuprocide 54Y (yellow cuprous oxide)	3 lb. + 1 qt. oil	do	30	3
Check	.....	do	27	.....
Bordeaux mixture	6–2	1941	81	58
Sodium polyphosphate (polyphosphates of sodium)	4 + 1 lb. lime	do	36	13
Copper oxychloride sulfate (copper oxychlorides and sulfates)	4 + 1 lb. lime	do	27	4
COCS 65 (copper oxychloride-copper basic sulfates)	6 lb.	do	39	16
Copper Hydro (copper basic salts)	5 lb.	do	25	2
Check	.....	do	23	.....
Bordeaux mixture	6–2	1942	62	41
Fermate (ferric dimethyldithiocarbamate)	3 lb.	do	44	23
Check	.....	do	21	.....
Bordeaux mixture	6–2	1943	51	.....
Fermate	3 lb.	do	37	.....
Fermate + Lime	3 lb. + 2 lb. lime	do	35	.....
Check	.....	do	73[a]	.....

TABLE 1.—(*Continued*)

Varieties and treatment	Amount of fungicide in 100 gal. of water	Year treated	Av. yield of nuts per tree	Increase or decrease in yield of sprayed trees over unsprayed checks
			lb.	*lb.*
Bordeaux mixture	6–2	1944	40	29
Check	.....	do	11	.....
Bordeaux mixture	6–2	1945	20	19
Dithane (disodium ethylene bisdithiocarbamate (dry))	3 lb.	do	5	4
Dithane, Zinc sulfate, and Lime	3–1-½ lb.	do	6	5
U. S. Rubber No. 604—Phygon (2,3 dichloro naphthoquinone 1,4)	3 lb.	do	3	2
Puratized N5-E (phenyl mercuri triethanol ammonium lactate)	1: 15,000	do	5	4
Puratized N5-E	1: 20,000	do	4	3
Isothane Q-15 (lauryl isoquinolinium bromide)	1 pt.	do	2	1
Check	.....	do	1	.....
Bordeaux mixture	6–2	1946	27	27
Bordeaux mixture + Omilite	1 qt.	do	17	17
Polyethylene polysulfide (Omilite)	2½ qt.	do	1	1
Puratized N5-E	1 qt.	do	4	4
337 (1-hydroxyethyl-2-heptadecyl glyoxalidine)	1 gal.	do	2	2
Do	2 gal.	do	3	3
341 (2-heptadecyl glyoxalidine)	1 gal.	do	1	1
Do	4 gal.	do	2	2
Check	.....	do	0	.....
Moore Variety				
Bordeaux mixture	6–2	1946	77	22
Bordeaux mixture + Dupont spreader-sticker	4 oz.	do	87	32
Copper A (2–5 $Cu(OH)_2$-$CuCl_2(CuO)_x$)	3 lb.	do	94	39
Copper A + Dupont spreader-sticker	4 oz.	do	92	37
Fermate	3 lb.	do	83	28
Fermate + Dupont spreader-sticker	4 oz.	do	83	28
Check	.....	do	55	.....

[a] Check trees accidentally sprayed one time—third application—with Bordeaux mixture.

year'' of the sprayed trees after these have had the stress of a heavy crop the preceding season. Furthermore, the daily distribution of rainfall was less than normal in 1943. This condition retarded the progress of the scab disease.

On the Moore variety in 1946 both Copper A and Fermate, with and without Dupont spreader-sticker, gave a greater increase in yield over the unsprayed check trees than did the Bordeaux mixture without the spreader-

sticker. This was probably because of the location of the trees, since the topography of the orchard prevented the randomization of the plots. However, the scab infection during the growing season, as well as quality of the nuts at harvest, indicated that there was no significant difference in the quality of the nuts from plots sprayed with Copper A, Fermate, or Bordeaux mixture, either with or without spreader-stickers.

In contrast to results with Copper A and Fermate, the addition of the spreader-sticker to the Bordeaux mixture increased the yield of nuts. The quality of the nuts was not affected, however.

The daily distribution of rainfall was above normal for 1946 at Albany, Georgia, but it was below normal at Monticello, Florida, which accounts for the high yield of nuts from the check trees at Monticello.

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NATURAL INFECTION OF REPLANTED APPLE TREES BY WHITE ROOT ROT FUNGUS

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(Accepted for publication October 10, 1947)

In the course of studies on root diseases of apple trees during the past 15 years the white root-rot fungus, *Corticium galactinum* (Fries) Burt, while relatively infrequent in occurrence, has been noted[2] to cause the death of many trees in the affected orchards. This paper records the results of replanting apple trees where trees affected with white root rot have been removed. It also discusses the source, persistence, and distribution of the infective material and the conditions favoring the infection of apple trees.

REPLANTING EXPERIMENTS

Replanting experiments have been carried on in 2 orchards where heavy losses from the disease have occurred. One of these orchards, located at Middletown, Virginia, in the Shenandoah Valley, contained 252 trees of the York Imperial, Grimes Golden, Winesap, and Stayman Winesap varieties that were 27 years old at the beginning of the experiment in the spring of 1937. This orchard was on land cleared of hardwood trees, predominantly white oak (*Quercus alba* L.).

The other experimental orchard, located at Heards, Virginia, in the Piedmont, contained 80 trees of the Winesap and Yellow Newtown varieties. This plot of 80 trees was part of a 50-acre orchard which was 18 years old in the spring of 1937 and was on land from which deciduous trees, predominantly chestnut and some oak, had been cleared.

At the beginning of the experiment there were gaps in the original stand of trees in both orchards. A detailed survey was made in 1937 to ascertain the number and location of the original trees killed by the white root-rot fungus. Two-year-old Delicious trees on seedling rootstocks were used for replanting both orchards in 1937, 1939, and 1940. Since the location of each original white root-rot case was known, the replanting experiments, it was believed, would yield pertinent information on the fate of young trees planted in foci of white root-rot infection.

In the replanting operations 3 methods were followed: (1) either 2 or 3 bushels of rotted manure were incorporated in soil in which the young tree was planted; (2) a 4-inch layer of coarse manure was placed at the bottom of the 20-inch-deep hole, a layer of soil was added, and the tree was planted and mulched with coarse manure; (3) the tree was replanted in soil without the addition of any manure.

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[2] Cooley, J. S., and Ross Davidson. A white root rot of apple trees caused by *Corticium galactinum*. Phytopath. 30: 139–148. 1940.

The results of the replanting tests are in table 1. Because of droughts and the lack of cultivation the replants had unfavorable growing conditions, and the mortality was greater than should be expected. The data recorded in the table show the condition of the original apple trees and of the replants in the Heards orchard in the summer of 1943 and in the Middletown orchard in 1944.

Tree growth was best where replanting method No. 2 was followed and poorest with method No. 1. In spite of variations in growth response, no relationship was found between replanting method and subsequent infection by the white root-rot fungus.

TABLE 1.—*Loss of replanted apple trees where white root rot is present in the orchard*

Original trees						Replants (set in 1937)		
Condition at replanting time in 1937			Condition in 1944			Condition in 1944		
Trees alive (per cent)	Trees dead from white root rot (per cent)	Trees dead from other causes (per cent)	Trees alive (per cent)[a]	Trees dead from white root rot subsequent to 1937 (per cent)[a]	Trees dead from other causes subsequent to 1937 (per cent)[a]	Trees alive (per cent)[b]	Trees dead from white root rot (per cent)[b]	Trees dead from other causes (per cent)[b]
Orchard at Middletown, Va.								
66.6	14.3	19.1	58.3	3.5	4.8	66.7	17.8	15.5
Orchard at Heards, Va.[c]								
58.7	15.0	26.3	48.7	5.0	5.0	54.5	27.3	18.2

[a] Based on original stand of 252 trees in the Middletown orchard and of 80 trees in the Heards orchard.

[b] Based on 84 replants in the Middletown orchard and 33 in the Heards orchard. Of the 84 replants in the Middletown orchard 36 were in white root-rot-infested sites, and of these, 15 died of white root rot by August, 1944. Of the 33 replants in the Heards orchard, 12 were in white root-rot-infested sites, and of these, 9 died of white root rot by July, 1943.

[c] Final record taken in 1943.

Further observations were made on the Middletown orchard in August, 1946, when more of the original trees had died of the disease. By this time some of the replants set where the original trees were affected with white root rot were beginning to bear. In other respects there was little change since 1944. The data indicate that apple trees set where white root rot was present are likely to succumb to the disease. As many as one-half such replants were affected by that disease in the period of 7 or 8 years. However, some of the trees that were set where the originial tree was known to have been removed because of white root rot have grown normally and seem healthy after 7 to 10 years. The best replants are where coarse stable manure was put in the bottom of the hole and covered with several inches of soil before the tree was planted.

SOURCE, PERSISTENCE, AND DISTRIBUTION OF INFECTIVE MATERIAL

Closely related to the replanting experiments and furnishing an understanding of some of the results is the study of the source, persistence, and distribution of infectious white root-rot material.

The observations made prior to and during the course of the replanting experiments (a period covering 15 years) help to explain some of these factors. At Middletown, Virginia, for example, the original planting grew satisfactorily until the trees were 15 to 20 years old, and then trees began to die on a clay ridge where the soil was poorer than in the rest of the orchard. The white root-rot fungus was not recognized as being pathogenic to the apple trees at the time, and so it can only be inferred that *Corticium galactinum* was responsible for the killing of the trees. In recent years the large and thrifty but now older trees growing in the better soil are dying of the disease.

Trees scattered over the orchard have continued to die of this disease during the time they have been under close observation. The disease usually attacks at the collar. The fungus, as shown by infected trees, does not radiate out from a focus of infection, as would be the case if the fungus spread through the soil, and this suggests dissemination by wind or manual means. When an apple tree is attacked by this pathogen it usually dies within 2 years after symptoms of the disease appear in its top, which discounts the probability that an affected tree is attacked many years before it dies. The trees in nearby apple orchards planted on land that was not newly cleared have not been affected by this disease. Nevertheless, the pathogen sporulates so freely that there is probably adequate wind dissemination of spores to give infection to nearby orchards, provided the proper substrate is present on which the fungus can become established. Inoculation experiments and observation of diseased trees indicate that for infection to take place in nature, it is necessary to have a reservoir of food on which the fungus can grow, such as woody material left on newly cleared land. Forest-tree stumps, and particularly the deeper roots, may serve for years as a substrate for the fungus; sporulation on diseased trees and their stumps probably provides inoculum for later infection of trees.

These observations indicate that the infection of apple trees by the white root-rot fungus is dependent upon woody material in the soil. This becomes inoculated with the fungus, and from this reservoir of infected material living apple-tree roots become infected. Where this woody debris is lacking, as in an orchard on old land, the fungus presumably fails to become established.

Searches have been made repeatedly for some wild host plant that is particularly susceptible to this disease. No evidence has been found that either the forest trees or the shrubby growth in the forest or other plants growing near an apple orchard are especially susceptible. On two occasions white-oak trees growing in proximity to an apple orchard were affected by

the disease, but in both cases the oaks were suppressed and also were growing in a poorly drained site.

Observations indicate that after a forest tree has been cut down the stump is susceptible to the attack of this fungus, which can live there as a saprophyte until the stump is rotted.

Leach[3] has noted that *Armillaria mellea* does not readily attack a vigorous living tree, but may readily attack a stump. A case has been observed by the present writer where ornamental shrubs were killed by *Corticium galactinum,* which apparently spread from an oak stump to the ornamental plants. At the same time that the shrubs were dying of the disease a black oak (*Quercus velutina*) was growing nearby in the infested soil and showed no evidence of being affected by the disease. The evidence at hand indicates that forest trees and shrubs are very rarely attacked by this fungus. It can, however, live as a saprophyte on stumps of various woody species and shrubby plants when growing in a soil containing inoculum.

SUMMARY

Replanting experiments have been carried on in 2 apple orchards in Virginia in which *Corticium galactinum* white root rot prevailed. Several times during the 8 years of the experiment the dead trees have been removed, the cause of the death of the removed trees determined where possible, other trees planted, and a record kept of the condition of the replant. As many as 15 per cent of the original apple trees of each experimental plot became affected with white root rot. The original trees, which ranged up to 37 years old, continued to die of the disease during the time the plots were under observation. During the period of the experiment, over one-half of the replants in infested soils became infected with white root rot.

Observations on the restricted distribution and the conditions favoring infection and spread of the disease are reported. The general deduction is made that the presence in the soil of woody material is necessary for the start and maintenance of the pathogen as well as for subsequent infection of apple trees.

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[3] Leach, R. Biological control and ecology of *Armillaria mellea* (Vahl) Fr. Trans. Brit. Mycol. Soc. 23: 320–329. 1939.

CYCLAMEN PETAL SPOT, CAUSED BY BOTRYTIS CINEREA, AND ITS CONTROL

C. M. Tompkins and H. N. Hansen

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INTRODUCTION

A petal-spot disease, affecting commercial varieties of potted Persian cylamen (*Cyclamen persicum* Mill.), has been observed each autumn and winter since 1935 in certain greenhouses in the San Francisco Bay region of California. The conspicuous symptoms decreased the market value of affected plants.

The results of inoculation tests and the method of controlling the disease are briefly discussed in this paper.

REVIEW OF LITERATURE

Although the gray mold disease of cyclamen plants, caused by *Botrytis cinerea* Pers. under conditions of high temperature and high humidity, is well known and frequently destructive on flowers, leaves, and petioles, it was not until 1938 that Wenzl,[1] in Austria, called attention to a hitherto undescribed petal-spot disease, also caused by *B. cinerea,* but prevalent in the cold, wet weather in late summer and fall of 1937.

SYMPTOMS OF THE DISEASE

Wenzl has adequately described and illustrated the symptoms of the disease affecting cyclamen petals, and hence only a brief, supplementary discussion is presented herewith.

The petal spots, at first mere flecks, soon enlarge to form round but more generally elliptical-shaped areas, 1 to 4 millimeters in diameter (Fig. 1 and 2). On pink, red, and salmon-colored varieties of cyclamen, the spots consist of an outer band of living tissue whose color is deeper than the normal color of the petal and a very small center which at first is of normal color, soon becomes water-soaked, and finally tan and necrotic (Fig. 1 and 2). On white-flowering varieties, the outer band of the spot first appears water-soaked while the center is necrotic; later the entire spot becomes necrotic. The spots, which may vary in number from a few to many, are scattered irregularly over the surface of the petal; usually between and with their long axes parallel to the veins. When heavily spotted, petals may be distorted.

All colored and white varieties of cyclamen grown as potted plants in greenhouses for the florists' trade appear to be susceptible when exposed to relatively low air temperatures (45° to 60° F.) and high humidities during the flowering season.

[1] Wenzl, Hans. *Botrytis cinerea* als Erreger einer Fleckenkrankheit der Cyclamen Blüten. Phytopath. Ztschr. **11**: 107–108. 1938.

FIG. 1. Flowers of a pink-flowering variety of Persian Cyclamen showing typical spots induced by *Botrytis cinerea* under natural conditions.

Leaf infection may occasionally be observed in conjunction with petal spot, but it is generally confined to older leaves and is of minor importance.

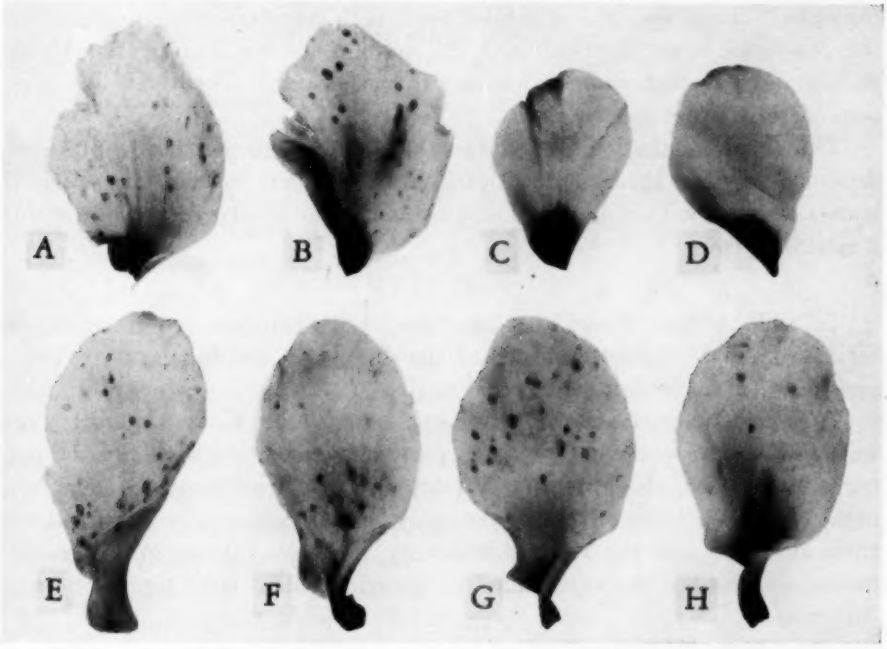

FIG. 2. Petals of a pink-flowering variety of Persian Cyclamen: A, B, E, F, G, H. Petals showing typical spots induced by *Botrytis cinerea* under natural conditions. C, D. Healthy petals.

THE CAUSAL FUNGUS, BOTRYTIS CINEREA

Each autumn and winter since 1935, unsuccessful attempts were made to isolate an organism from spotted petals by means of tissue plantings on poured agar in Petri dishes and by placing infected petals in moist chambers. Finally, in 1945, pure cultures of *Botrytis cinerea* were obtained with ease on poured plates and in moist chambers. No valid reason can be given for failure to isolate this fungus in earlier trials.

Healthy white- and colored-flowering varieties of cyclamen were inoculated in a cool greenhouse during the fall and winter months of 1945 and 1946. The potted plants, on saucers, were given a thorough watering late in the afternoon to provide the necessary humidity. Then the upper and lower surfaces of partly and fully expanded petals were atomized with spores of the fungus suspended in sterile-distilled water. Controls were atomized with sterile-distilled water, after which all plants were covered with glass jars. In 1945, a total of 10 white and 16 colored potted cyclamen plants, each with 6 to 10 or more flowers, were inoculated, while a corresponding number of plants served as controls. All petals sprayed with the organism became infected in less than 15 hours, while the controls remained healthy. The symptoms on artificially-infected petals were identical with those of naturally-infected specimens. In 1946, 6 white- and 12 colored-flowering varieties were inoculated; controls consisted of the same number of plants. The results were comparable to those obtained in the preceding tests. The fungus was reisolated from each set of inoculated plants, and the reisolates were identical with the parent culture and proved highly pathogenic. No infection of leaves and petioles was observed while these tests were in progress.

The short incubation period for the disease under experimental conditions is in close agreement with observations made in commercial greenhouses where rapid development and spread of the disease may occur within a matter of hours.

CONTROL OF THE DISEASE

In 1938, before Wenzl's[2] publication was available and prior to the establishment of the true nature of the disease in California, an effective and practical method of control was devised.

It is customary, locally, to grow cyclamen plants in continuously cool, well-ventilated greenhouses. When plants commence to flower in September and October, the humidity is relatively low, and they are free from petal spot. With the advent of the rainy season in November, air temperatures decrease and the relative humidity increases. During the evening hours, apparently the high humidity provides ideal conditions for rapid infection.

The disease may be controlled by changing the environmental conditions surrounding the plants during the evening hours. It was found that by

[2] See footnote 1.

closing the top or peak ventilators of the greenhouse at dusk, leaving the side ventilators wide open, and turning on the steam in each of two pipes on opposite sides and traversing the length of the greenhouse, the disease can be entirely prevented. While the heat thus provided is sufficient to reduce the relative humidity to a point unfavorable for infection, it does not materially increase the air temperature. Early the next morning, the top ventilators are opened wide and the steam is turned off. This method then becomes a standard daily practice. Apparently air temperature is of secondary importance, because the disease which has been observed over a wide range of low temperatures may easily be controlled by providing a drier atmosphere.

This method of control has no effect on previously-infected petals. They should be excised from the plants before adopting the method described above in order to observe the full effects on the new flower buds as they open. New petals are always free from spotting.

SUMMARY

Petal spot of Persian cyclamen is prevalent in the late fall and winter months, coinciding with the rainy season, in greenhouses in the San Francisco Bay region of California.

Symptoms of the disease consist of small spots, round to oval, few to many, scattered at random between the veins of the petal. With colored varieties, the spots contain an outer area of deeply-colored tissue with a small, tan, necrotic center. On white varieties, the outer area is at first water-soaked, with a tan, necrotic center; later the entire spot becomes necrotic. No leaf infection occurs.

The disease, first recorded by Wenzl in Austria, is caused by *Botrytis cinerea*.

Healthy cyclamen plants were artificially infected in the greenhouse by atomizing with a spore suspension of the fungus. The incubation period on the petals was less than 15 hours.

The disease is favored by relatively high humidity, accompanied by low air temperatures (45° to 60° F.).

Prevention of the disease depends upon daily closing of the top ventilators of the greenhouse at dusk, leaving the side ventilators wide open, and turning on two steam pipes to provide drier air conditions during the evening hours.

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A PHYCOMYCETE PARASITIC ON APHIDS

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(Accepted for publication October 13, 1947)

In the potato growing areas of the State of Maine, aphids are the principal means of spreading virus diseases. During detailed studies on the problem of control of the various virus diseases attention was given to the abundance of aphids. It was observed that frequently fungi were apparently the cause of considerable mortality among the aphids, and an effort was made to isolate the species of fungi present on dead aphids. A collection of fungus-infested aphids was made near Mapleton, Maine.[2] The diseased insects were found on the underside of the older potato leaves. There were four species present: the green peach aphid, *Myzus persicae* (Sulz.); the potato aphid, *Macrosiphum solanifolii* (Ashm.); the foxglove aphid, *Myzus convolvuli* (Kltb.) (*pseudosolani* Theob.); and the buckthorn aphid, *Aphis abbreviata* (Patch). The dead insects were cultured and the fungi isolated were held for study. After the field season, observations on aphids parasitized by fungi were continued in the greenhouse. Isolations of the fungi found on aphids growing on greenhouse plants furnished additional cultures for study. It was during the course of this work that the fungus described in this paper was isolated and later identified.

METHODS AND MATERIALS

In isolating the fungus dead aphids were placed on water agar on the bottom of a sterile Petri dish. Over each insect was placed a sterile Van Tieghem cell. Entomophthorous fungi are known to be phototropic, so the Petri dishes were placed in a cardboard box with light admitted through a small hole in the top. Within a few hours the fungus began to discharge spores against the lower surface of the cover slip on top of the Van Tieghem cell. Within 24 hours the spore mass was dense enough to be seen with the naked eye.

Masses of spores were transferred from the cover slip to four types of media. An effort was made to provide media containing some form of protein. Sawyer (7) working with fungi parasitic on the black-headed fire worms *Ropobota vacciniana* found that among other things the yoke of eggs from which fat had been extracted by ether was an excellent medium for growth. A quantity of such medium was prepared and spores transferred under sterile conditions. Growth was slow with little aerial mycelium but numerous spores were produced. A second medium was made by adding three gm. beef extract and ten gm. gelatin to a liter of water agar. On this

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[2] Grateful acknowledgment is made to W. A. Shands for his aid in locating potato fields having diseased aphids and his general interest in the study; and to Mrs. Flora G. Pollack for identification of the fungus and for literature citations.

medium the fungus made a slow growth. Later, a third medium was made using ten gm. dextrose in addition to the other ingredients of the second medium. The fungus still made a slow growth. A fourth medium used was malt agar. The fungus grew and sporulated vigorously. Spores were cast in the direction of strongest light and completely covered the under surface of a Petri dish top in a few days.

IDENTIFICATION OF THE FUNGUS

A study of the fungus in culture showed that the mycelium in the medium was much branched but the aerial hyphae were usually unbranched.

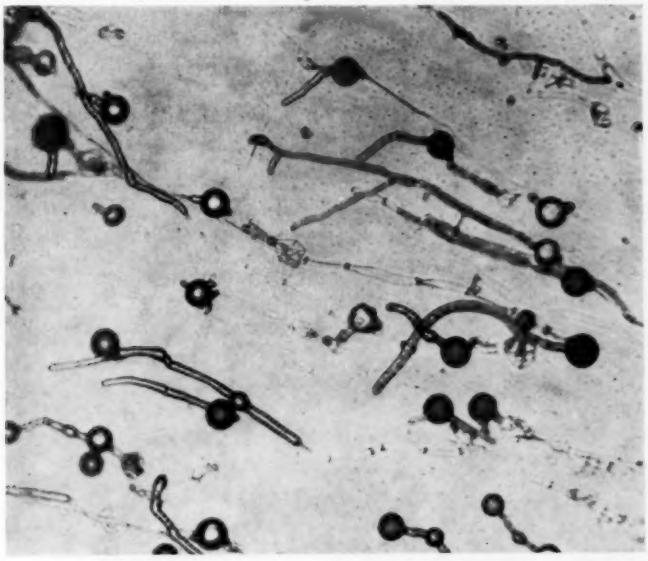

FIG. 1. Germinating conidia of *Entomophthora coronata* showing papillae, secondary conidia, and segments of mycelium both filled with protoplasm and empty. ×100.

The hyphae were rather large and averaged about 12 μ in diameter. The conidiophores were mostly unbranched and erect. They were positively phototropic and similar to the mycelium in size and shape. The conidia were rather large and globose, with a prominent papilla at the base: they averaged about 30 μ in diameter. Under humid conditions and at room temperature, the conidia germinated very easily to give rise to mycelium or secondary conidia (Fig. 1). In old cultures, a number of conidia which behaved as resting spores were observed. These conidia had slightly thicker walls and were somewhat darker than young conidia but their most outstanding characteristic was a covering of blunt hairlike appendages over the entire surface (Fig. 2). Resting spores which had germinated produced conidia similar in appearance to those produced by conidiophores above the mycelial layer. In old cultures the hyphae produced septae making segments which were either empty or filled with protoplasm. Those with proto-

plasm were observed to enlarge somewhat and to take on the appearance of a hyphal body which sometimes resumed growth when conditions were favorable.

This fungus was first observed in the United States by Martin (6) who cultured it out of decayed wood. He applied the name *Conidiobolus villosus*, based on the fact that the genus *Conidiobolus*, founded by Brefeld (1) was distinguished from *Entomophthora* because it is a saprophyte. Later work on the morphology and physiology of the fungus by Kevorkian (5), who found it on living termites in Cuba, showed that it was a parasite as well as a saprophyte. Kevorkian made a critical study of the morphology

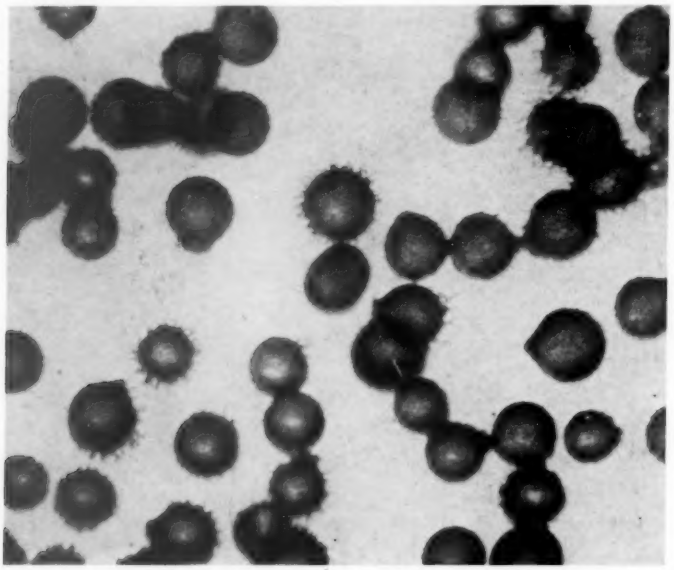

FIG. 2. Conidia of *Entomophthora coronata* in resting stage, covered with blunt hair-like appendages. ×300.

and physiology of the fungus he isolated from termites together with the fungus isolated by Martin. He noted that a similar fungus was described earlier by Gallaud (4), who found spinose resting spores. Constantin (2) identified this fungus as *Delacroixia coronata* (Cost.) Sacc. Kevorkian concluded that the parasitic nature of the organism, not observed by Martin, together with its morphology as described by earlier workers warranted a new combination. He applied the name *Entomophthora coronata* (Cost.) Kevorkian. A study of the fungus found by the writer on the green peach aphid in the greenhouse shows that it is identical with that studied by Kevorkian and because of its parasitic habit should be described as *Entomophthora coronata*. This fungus was also found along with *Empusa aphidis* Hoff. in a collection of dead green peach, potato, and foxglove aphids from a potato field near Mapleton.

INOCULATION STUDIES

In order to determine whether the fungus was pathogenic on the green peach aphid a method of natural inoculation was devised. Cultures of the fungus were grown on malt agar in 50-mm. Petri dishes. When the fungus was sporulating freely cultures were placed directly under potato leaves supporting a heavy aphid population. The Petri dishes were so placed that light came to the fungus through the potato leaf. The fungus cast numerous spores against the lower surface of the leaf. Most of the aphids moved to some other portion of the plant, but there were a small number of aphids remaining which were diseased or dead. Upon being cultured the fungus was recovered.

A second method of exposing aphids to the fungus was devised. Potato leaves were cut from a plant and all but the terminal leaflet removed. Each leaflet was infested with a large population of aphids. The stem was placed in water and the leaflet placed over a Petri dish in which was a vigorously sporulating culture of the fungus. After being exposed to the fungus spores for two days, the aphids were transferred to a potato plant previously free of aphids. The following day and for several days thereafter a number of dead aphids were found on the under side of the potato leaves. Upon being placed on maltose agar they yielded cultures of the fungus. Although a large number of aphids were exposed to the fungus a relatively small proportion of them became infected.

A number of aphids that had been exposed to the fungus were studied under magnification sufficient to enlarge the aphid ten times. Spores were observed clinging to various portions of the aphid body. Since the fungus shows a preference for carbohydrates as a growing medium and since the aphids secrete a carbohydrate substance known as honeydew it seems possible that the fungus may grow into the body of the aphid on food material produced by the insect.

DISCUSSION

The biological control of the aphids in potato fields by means of fungi offers an interesting field of investigation. Dustan (3) has shown that biological control of an insect by means of a parasitic fungus is possible through artificial culture and dissemination of the spores. The results of studies reported in this paper indicate that *Entomophthora coronata* can be cultured rather easily, but only a small percentage of aphids exposed to it become infected. Field observations indicate that the fungus usually does not appear until the aphid population on a plant is very high. Under field conditions this fungus together with other species of entomogenous fungi, given favorable environmental conditions, have destroyed aphids on potato vines late in the season after the aphids were very numerous. By introducing this and other species of entomogenous fungi into potato fields before such fungi normally appear, these studies suggest that aphids possibly could

be infected. An epidemic of fungus infection on the aphids might be started at a period early enough to have a possible value in aphid control.

SUMMARY

1. Under greenhouse conditions, a fungus, *Enthomophthora coronata* (Cost.) Kevorkian has been found to be parasitic on the green peach aphid.

2. The fungus was grown on media containing both proteins and sugars. Best growth was observed on maltose agar.

3. Inoculation experiments suggest that the fungus is pathogenic on aphids.

4. A study of living aphids exposed to the fungus indicates that spores present on the body of the insect may germinate on honeydew secreted by the insect.

5. The study suggests that this fungus may have some value as a natural agency of aphid control.

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PHYTOPHTHORA CINNAMOMI ROOT ROT OF AVOCADOS UNDER TROPICAL CONDITIONS[1]

BOWEN S. CRANDALL[2]

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INTRODUCTION

In 1944, soon after the start of organized pathological work at the Estacion Experimental Agricola de Tingo Maria and about 8 years after the opening of the region to colonization, dying and declining avocado trees, *Persea americana* Mill., were observed. The first cases were large, mature trees located on the properties of small landowners. These trees showed clear cases of collar rot followed by quick death and from them *Phytophthora cinnamomi* Rands was isolated. Later the same year the disease was found in seedling and mature avocados being grown by the Station and *P. cinnamomi* was again isolated. Apparently the first recognition of the disease in this area, at least in this part of South America, was reported in 1945 (3). The disease is apparently the limiting factor to avocado growing in this region. Because of the controversial nature of the evidence presented by various workers on the relationship between *P. cinnamomi*, wet soil, and the decline or collapse disease, it was necessary to undertake sufficient work to solve the problem locally, in order that recommendations could be made in regard to the avocado program. The results herein reported are based on observations and experiments on trees growing under conditions of high rainfall and often on poorly drained soils. The work was designed to determine whether *P. cinnamomi* is the primary cause of the decline or collapse disease, and if so, whether a resistant root stock can be found or methods of planting or site selection be used to eliminate the disease or hold it to a minimum.

HISTORY OF THE DISEASE

Avocado root rot caused by *Phytophthora cinnamomi* was first reported from Puerto Rico in 1929 by Tucker (10, 11). These first reports showed that conditions of waterlogging and *P. cinnamomi* had to be present to cause the disease. Wager (12) in 1940 reported the disease in California and expanded the experimental work demonstrating the connection between conditions on poor soil drainage and ability of the parasite to cause disease. Specific experimental evidence was presented showing that avocados grow-

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ing in pots could be artificially infected with *P. cinnamomi* and disease symptoms produced if the pots were submerged for a period as short as 2 or 3 days, while no infection or disease symptoms would appear in trees given normal watering. In 1941 Wager (13) reported the disease from Western Transvaal and Natal in South Africa. Field observations stressed the appearance of the disease on trees growing in heavy clay soils or on poorly drained sites. Stevens and Piper (9) reported what was later determined to be the same disease from Florida in 1941. Wager (14) in 1942 amplified his earlier work demonstrating the relation between wet soil and the disease.

An open discussion on decline of avocado trees on wet soil was reported in the 1937 California Avocado Association Yearbook (1). It seems evident that the problem under discussion was the root disease caused by *Phytophthora cinnamomi*, then unknown in California. The suggestion that waterlogging was causing the decline was questioned when it was brought out that the avocado came originally from regions of high rainfall. Various reasons were advanced to support the argument that waterlogging caused the disease, among them that in the tropics the trees grow on well drained soils or that they are adapted to the local condition. A heavy loss observed in Mexico after an unusually wet season was mentioned and the suggestion made that sudden change could be disastrous. Decline was again discussed, by Rounds (8) in the 1939 Yearbook of the Association, and also, in the 1941 Yearbook (2) when Wager's work (12) was mentioned. In 1943 Klotz and Sokoloff (6) tentatively expressed the view that *P. cinnamomi* and other pathogenic fungi might attack the roots after they were predisposed by injury following waterlogging. Parker and Rounds (7) in 1943 showed the relation of soil moisture and drainage to decline of mature avocado trees and while citing work such as Wager's (14) in relation to soil moisture and decline did not mention the connection of *P. cinnamomi* with this decline. In 1944 Harvey (5) reported the results of a survey made in California by the Emergency Plant Disease Prevention Project to determine the incidence of *P. cinnamomi* in relation to avocado decline. He isolated *P. cinnamomi* from the roots of 164 out of 268 healthy appearing trees growing in close proximity to declining trees. Zentmyer, Klotz, and Miller in 1945 (17) presented experimental evidence that tends to show that avocados decline in flooded pots regardless of the presence or absence of *P. cinnamomi*. Zentmyer and Klotz in 1947 (15) stated that *P. cinnamomi* may be a primary factor in decline of avocado trees but later the same year (16) stated that poor drainage generally initiates the trouble. They further report that waterlogging and *P. cinnamomi* appear to be involved in decline and that trees in waterlogged soils will decline without the fungus being present but that the presence of *P. cinnamomi* will accelerate the decline.

The relationship between soil moisture, drainage and *P. cinnamomi* is recognized on other hosts. Crandall, Gravatt, and Milburn (4) showed

the relationship of the recession of the American chestnut and chinkapin with conditions of soil drainage and also report a number of nursery diseases of various hosts clearly linked with predisposing conditions of flooded soils. The relationship between high moisture conditions and parasitism by the genus *Phytophthora* in general is well known.

GEOGRAPHICAL DISTRIBUTION

Phytophthora cinnamomi is not listed in La Flora Fungosa Peruana by García Rada and Stevenson published in 1942, on avocado or other hosts. The writer has heard verbal accounts of epidemic losses of avocados from root rot in other regions of Peru which occurred some years ago and that could have been caused by *P. cinnamomi*. However, so far as has been determined, the disease is present only in the Tingo Maria region. There seems no reason to suppose, however, that it may not be of widespread occurrence on avocados wherever they are grown under environmental conditions favorable to the fungus.

SYMPTOMS OF THE DISEASE

In this region two distinct methods of attack have been observed on both seedling and mature trees. The first is characterized by appearance of chlorosis followed shortly by sudden wilting and death (Fig. 1). The second

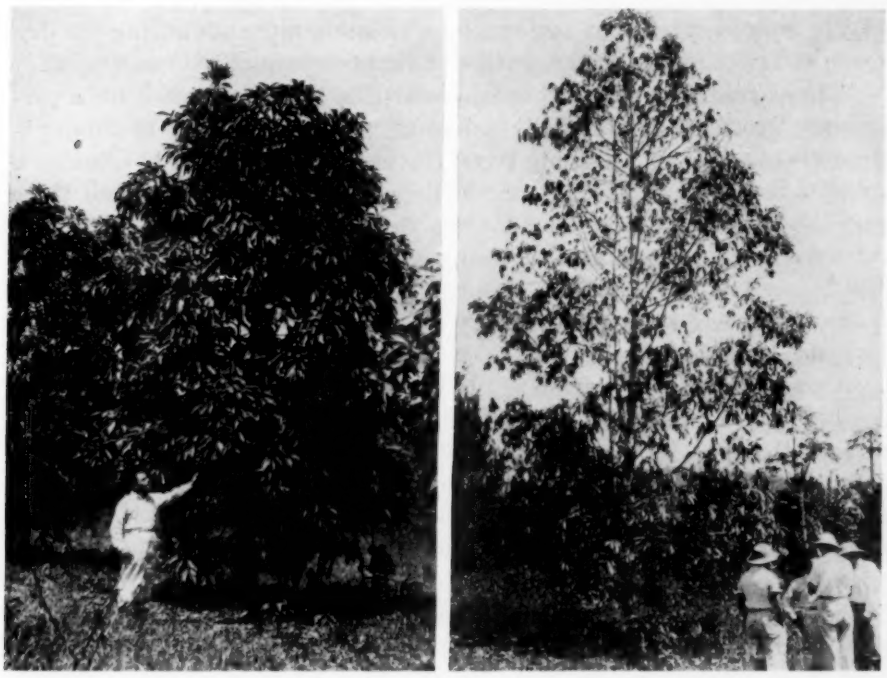

FIG. 1. Healthy appearing avocado tree on left contrasted with, right, avocado wilting as a result of canker at the collar.

is a slow decline of the trees characterized by die-back of the branches and often typical drought symptoms in the leaves. In small trees with slow decline symptoms, death usually follows during the same season but mature trees seem to partially recover during the dry season. Some trees under observation for three years, are still in little worse condition than when first observed.

Light brown girdling cankers in the cambium and phloem tissue at the collar region and immediately below are found on trees showing chlorosis and sudden wilting. The advancing margin of the infection extending into healthy tissue is irregular and wedge shaped. The surface of this infected area is black or darker brown than the surrounding healthy bark. Isolated islands of infection and dead or infected small rootlets may be present or absent. In contrast, trees showing slow decline, either completely lack the infection at the collar or it is present on one side or portion of the collar only. The small rootlets are dead or infected, and islands of infection may be present or absent on the lateral and main roots.

ISOLATION AND INOCULATION

During the wet season *Phytophthora cinnamomi* appeared in all of the tissue cultures from the active cankers in the collar region, in 97 per cent from the smaller rootlets, in 18 per cent from the larger roots, and in 9 per cent from the islands of infection on the larger roots when special techniques for Phytophthora isolation were used.[3] Isolation attempts during the dry season and isolation by routine methods have been uniformly unsuccessful.

Six one-year-old criollo avocados growing in boxes were used for a preliminary proof of pathogenicity following the first isolation of *Phytophthora cinnamomi* from a dying tree. Three were inoculated by placing a mycelial mat of the *Phytophthora* in the soil against the collar and three were left uninoculated as checks. The trees were not watered artificially but were left outside during the rainy season. The three inoculated trees died before the end of the wet season while the three check trees were still healthy at the end of the following dry season. *Phytophthora* was recovered from the dying inoculated trees.

A project was set up primarily to test the resistance, by inoculation, of the different races of avocado. In October, 1945, the Station received, through the U. S. Bureau of Plant Industry, Office of Foreign Plant Introduction, 100 seeds of the Gottfried variety (Mexican race), 39 seeds of the Family variety (an old Florida selection and probably from a cross of the West Indian and Mexican races), and 32 seeds of West Indian seedling selections. Eighty-two of the Gottfried variety, 11 of the Family, and 10 of the West Indian seeds germinated and produced healthy plants. These were used with 130 criollo avocados, 19 of which were reserved as uninoc-

[3] These methods principally consisted of placing carefully selected sections in sterile water blanks for periods varying from 3 to 5 days with frequent changes of water and then lightly surface sterilizing before planting on cornmeal agar.

ulated checks in a separate bed. The plants were in well drained beds of friable clay-loam and the varieties were separated by drainage canals. The plants were inoculated in late January and early February by placing mycelial mats of *Phytophthora cinnamomi* against the collar of each tree. Two months later 80 per cent of all inoculated plants were dead or dying, and before the end of the dry season in September a 100 per cent loss had occurred.

Phytophthora cinnamomi was isolated from the dying plants. The uninoculated check plants showed no symptoms of disease throughout the wet season and the following dry season. By January, 1947, they uniformily showed symptoms of root rot and quickly declined. *P. cinnamomi* was isolated from these plants.

EPIDEMIOLOGY

Tingo Maria is at an elevation of approximately 2200 feet on the Huallaga River, a tributary of the Amazon in the Peruvian montaña. The year is divided into rather distinct wet and dry seasons. Records since 1940 show an average rainfall for the 6 dry months of April through September of 43.25 inches, and of 91.66 inches for the 6 wet months of October through March. The driest month was July, 1943, with 1.2 inches, and the wettest month was December, 1945, with 25.8 inches. The wet season of 1940–41 had the most rainfall, a total of 106.7 inches for the 6-month period. The 1944–45 wet season had 78.6 inches and the 1945–46 wet season 99.1 inches. Soil pH of avocado sites ranges from 4.5 to 7.0.

Losses from root disease have been more noticeable either at the beginning or toward the end of the wet season, probably because symptoms are more in evidence at these times. The disease apparently appears at any time during the wet season and with the exception of trees parasitized during the wet season but dying after the onset of dry weather, is apparently inactive during the drier months.

Avocados are not grown in the region in large plantings. The majority of the small land owners have a few trees each to produce fruit for home use and limited local sale. The bulk of these trees are criollo seedlings. No losses of epidemic proportions in any given area have occurred. From time to time a loss of one or two isolated trees is observed. In almost every case observed of trees developing chlorosis and sudden wilting, healthy trees remain on closely adjacent sites. Individuals and small groups have died on poorly drained sites but healthy individuals remain on adjacent equally poorly drained sites. Likewise similar losses on well drained sites have been adjacent to apparently healthy trees. Examination of the root systems of these apparently healthy trees has disclosed that the roots and rootlets were generally in good condition, although some rootlets were dead and some isolated cankers were present. The direct cause of the wilting and death were girdling cankers at the collar. *Phytophthora cinnamomi* was isolated from the girdling cankers.

Only one instance of what might be called slow decline or die-back has been observed. This is in a grove of mature and semi-mature trees located on the grounds of the Experiment Station. This group presents in greater or less degree, the die-back and decline symptoms. Only a few have died and the condition of the remainder appears little worse now than 3 years ago. At least partial recovery seems to take place during each dry season. Partial excavation of the root systems has shown a surprising lack of living small rootlets. *Phytophthora cinnamomi* has been isolated from the dying rootlets on these trees. The trees are growing on a site which is well drained but with heavy clay soil.

During the current wet season symptoms of drought in the leaves and die-back appeared in beds of seedling avocados being grown for use as budding stocks in the same general area but on a loamy soil. Higher and better drained parts of the beds were at first unaffected. Examination of the seedlings with drought symptoms and die-back showed that almost all the small rootlets were dead or dying. No collar infections were found. *Phytophthora cinnamomi* was isolated from a high percentage of the dying small rootlets.

CONCLUSIONS

Studies of the disease made in this region, under environmental conditions which are probably normal for the avocado, perhaps give a clearer picture of the disease as a whole than studies made in regions where the tree has been introduced and is growing under abnormal conditions. Any controversial issues of the role of pathogen versus wet feet do not seem to be important where the tree is growing normally under rather wet conditions. The reported experiments which tend to show that *Phytophthora cinnamomi* is a pathogen only when the tree is subjected to conditions of poor drainage, actually probably only reproduce conditions which often occur in regions where the tree is in its normal environment.

Viewed in relation to the appearance of this disease in the Tingo Maria region the various views previously summarized do not present a controversial issue. Each presents a portion of the picture and the sum total seems merely to show that under certain conditions the disease is not a factor unless soil conditions approach those often normally present in the tropics during the wet season. Undoubtedly trees grown under dry conditions with root system developed for these conditions would suffer if the environmental conditions change toward the wet side. Likewise such trees would then be growing under conditions ideal for attack by *Phytophthora cinnamomi*.

Seen under local, normal conditions this disease does not appear different, except possibly in degree, from other root diseases caused by Phytophthoras. Like many such Phytophthora-induced diseases, conditions of high soil moisture are usually required before the fungus parasitizes its host. Whether these conditions constitute predisposing conditions by adversely

affecting the host or whether they only create the conditions necessary for growth of the fungus is beside the point. Probably, dependent upon the amount of shift of the environmental conditions away from the optimum for the host and toward that of the parasite, both occur. The writer personally feels that in this and other tropical and subtropical Phytophthora-induced root diseases the effect of the change toward wetter environment is more important to the fungus parasite than the host. Improvement of planting techniques and site selection with the objective of better soil drainage have given beneficial results in the case of this disease as well as others, when a susceptible host must be grown in the presence of a Phytophthora parasite.

In the face of this disease, widespread planting of avocados is not being encouraged in this zone. For such trees as are planted only the better drained sites on sandy or loose, friable soils are recommended. The uniform susceptibility of the avocado races thus far tested indicates that development or selection of a resistant root stock is not a likely possibility unless some resistant parent type exists. Search is still being made for such a type.

SUMMARY

Phytophthora cinnamomi, root rot of avocados, has been found in the Tingo Maria region on well and poorly drained soils, on light and heavy soils, and on mature and nursery age trees. In all cases examined, healthy trees were growing on the same sites and under similar conditions but without disease. Two manifestations of the same disease have been studied. Mature and nursery age trees which develop chlorosis and sudden wilting (what apparently is called quick decline in California) are infected at the collar region with girdling cankers. Slow decline and die-back on both mature and nursery age trees apparently are caused by infection and death of the smaller rootlets, with the main roots and collar generally free from infection. *Phytophthora cinnamomi* has been isolated from both types of infections. The pathogenicity of the strains isolated has been demonstrated by artificial inoculation on trees growing under normal wet season conditions, with symptoms of chlorosis and sudden wilting. The fungus has been recovered from inoculated trees following the appearance of the symptoms. Uninoculated check trees, growing under identical environmental conditions, have remained healthy.

U. S. DEPARTMENT OF AGRICULTURE
AND
ESTACION CENTRAL DE COLONIZACIÓN,
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GLOEOSPORIUM DECAY IN GRAMINEAE[1]

RODERICK SPRAGUE[2]

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In an earlier report (7), *Gloeosporium sp.* was mentioned as present on the roots of various grass and cereal hosts in North Dakota but, except for one instance, it had proved non-parasitic. Recently the writer (8) reported that several isolates of a carrot-red, mucose *Gloeosporium* caused seed rot of various plants in sterilized soil. On the basis of this work, further study was made with the *Gloeosporium* that produces carrot-red masses of conidia on potato-dextrose agar and also with a common strain that develops pink masses of conidia on the same medium.

HOST RANGE AND GEOGRAPHICAL DISTRIBUTION

This fungus has been isolated from 121 species of cereals and grasses and its host range on this family appears to be virtually unlimited. It would appear, therefore, to be unnecessary to list so lengthy an array of hosts in this article. All cereals and common species of grasses in North Dakota harbor the fungus.

This species has been isolated from field material collected in Minnesota, North Dakota, South Dakota, Nebraska, Wyoming, Montana, and eastern Washington, and it probably occurs over a far wider area.

PURE CULTURES

The fungus is readily isolated on potato-dextrose agar from water agar plates of water-washed roots and crowns. The isolates grow very slowly at first but eventually they cover test tube slants with yeasty masses of conidia. The isolates, which form pink spore masses, first produce pale cream-colored colonies. These soon become bright pink masses of conidia that eventually germinate to form black smooth or wrinkled carbonaceous colonies. Sometimes pycnidium-like stromata are formed. On sterilized sand plus 0.85 per cent bran and 0.5 per cent dextrose, the colonies are gray, cottony, and composed of true mycelium. Sometimes chlamydospores similar to those formed by *Fusarium oxysporum* (Schlecht.) em. Snyder and Hansen occur.

The isolates that produce carrot-red masses of conidia are possibly somewhat slower in forming black stromatic hyphae. There are a few isolates

[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, Soils, Fertilizers and Irrigation, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Nursery Division, Soil Conservation Service, United States Department of Agriculture and the North Dakota and Washington Agricultural Experiment Stations. Published as Scientific Paper No. 736, College of Agriculture and Agricultural Experiment Station, State College of Washington, Pullman, Wash.

[2] Formerly pathologist, Division of Cereal Crops and Diseases and collaborator. Division of Forage Crops and Diseases.

that are intermediate in color between the pink and the carrot-red types while there are still other isolates that scarcely sporulate at all and resemble cultures of *Phoma*.

In an earlier article (7) the writer reported 919 pure cultures of *Gloeosporium* out of a total of 22,832 cultures of all fungi isolated up to that time. *Gloeosporium* thus constituted about 4 per cent of the total. In 1944, *Gloeosporium* averaged 6.7 per cent of the total while during the cool season of 1945, it averaged over 14 per cent of that year's isolates. During mid-January, 1946, 26 per cent of the isolates from dormant grasses were referable to *Gloeosporium* (8). In these January isolates of *Gloeosporium*, 96 were the pink strain while 52 were the carrot-red one. The pink strain usually has been the more common one, particularly during the summer months. Both strains, however, were isolated from necrotic roots during all months of the year. In 1947, a dry season, only 1 per cent of the isolates from the roots of Gramineae at Pullman, Wash., were *Gloeosporium*.

ARTIFICIAL INOCULATIONS

This species appears to be one that performs erratically under artificial inoculation conditions. Inoculum was increased on the sand-bran-dextrose formula under aseptic conditions in milk bottles and added one inch beneath the seed in electrically sterilized soil in $14 \times 20 \times 4$ inch flats in the greenhouse. The temperature was variable, ranging from 55° to 80° F. during the latter part of the winter of 1945–46.

The results of all recent inoculation trials with *Gloeosporium* are summarized in table 1. Percentage loss was calculated the same as in earlier trials (7). Seven tests with the pink isolates showed two parasitic isolates, two that were questionable, and three that were non-parasitic. Of the carrot-red isolates, five were positive, one questionable, and two negative. Of the five carrot-red isolates that were positive, however, only three were original field isolates while the other two were reisolates from one of the original positive cultures.

All of the results indicate that *Gloeosporium* is short lived under artificial greenhouse conditions. Apparently saprophytic contaminants (mostly bacteria) soon suppress it in these greenhouse trials. Before the fungus is suppressed, however, it sometimes causes complete pre-emergence death of the seed of some hosts such as blue grama or alfalfa. Plants with larger seeds such as corn, beans or pumpkins, had sufficient food reserve to permit the seedlings to survive and recover. In one case, however, even pumpkins were not only retarded but the seed rotted. Sometimes wheat had all the roots stubbed off by *Gloeosporium* so that the stunted culm and the attached brown, decayed seed were readily lifted from the flat.

As mentioned, infection is very erratic with the methods used and therefore no attempt was made to distinguish race differences. For this, it probably will be necessary to work out a technique similar to that used by Jaarsveld (3) with *Rhizoctonia solani*.

TABLE 1.—*Results of inoculating sterilized soil at seeding time with sand-bran-dextrose cultures of Gloeosporium sp. in the greenhouse at Mandan, N. Dak., 1941–1946*

Culture No.	Original Host	Color of Strain	Percentage loss due to rot on:																	
			Alfalfa	Beans, Field	Beets, Garden	Bluegrass, Ky.	Brome, Smooth	Cabbage, Chinese	Corn, Field	Grama, Blue	Grass, Pigeon	*Hordeum brevisubulatum*	Oats, Common	Proso, Turghai	Pumpkin, Pie	Sorghum, Amber	Tomato, Rosana	Wheat, Common	Wheatgrass, Standard Crested	Wheatgrass, Intermediate
53C–1	Brome, Smooth	Carrot-red	.....	.....	.....	0	0	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	0	0	.....
56B–4	Brome, Smooth	Pink	.....	.....	.....	.....	tr	.....	.....	0	.....	.....	0	.....	.....	.....	.....	?4	?3	.....
Check	.....	.....	.....	.....	.....	0	0	.....	.....	0	.....	.....	0	.....	.....	.....	.....	0	0	.....
3701A–6	*Elymus excelsus*	Carrot-red	96	50	29	2	.....	92	tr	40	78	29	5	68	1	7	20	28	32	30
49C–1	Grass, Pigeon	Pink	.....	.....	.....	0	0	.....	0	.....	.....	.....	0	0	.....	.....	.....	0	0	.....
116A–1	*Muhlenbergia japonica*	Pink	.....	.....	.....	0	0	.....	.....	.....	.....	.....	.....	0	.....	.....	.....	0	0	.....
2613A–1	Timothy	Pink	100	.....	.....	50[a]	.....	.....	32	93	.....	.....	13	98	.....	24	.....	65	65	.....
2649A–5	Wheat, Common	Pink	0	.....	.....	0[a]	.....	.....	0	30	.....	.....	0	0	.....	0	.....	0	tr	.....
5B–1	Wheatgrass, Crested	Intermediate	.....	.....	.....	0	0	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	0	0	.....
217A–4	Wheatgrass, Crested	Pink	.....	.....	.....	0	0	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	0	0	.....
1740A–7	Wheatgrass, Crested	Carrot-red	0	.....	.....	0[a]	.....	.....	0	40	.....	.....	0	0	.....	0	.....	0	0	.....
2624A–1	Wheatgrass, Crested	Pink	100	.....	.....	5[a]	.....	.....	6	64	.....	.....	0	55	.....	40	.....	tr	10	.....
3200B–1	Wheatgrass, Intermediate	Carrot-red	0	.....	.....	0	.....	.....	0	0	.....	.....	.....	0	.....	0	.....	0	0	.....
3263A–1	Wheatgrass, Intermediate	Carrot-red	26	12	.....	9	.....	82	tr	8	6	2	tr	16	35	tr	50	4	14	0
3257A–2	Wildrye, Canada	Carrot-red	84	60	36	40	0[b]	75	?2	69	.....	25	0	7	0	tr	0	4	10	4
3705A–1	Reisolation of 3257A–2	Carrot-red	100	60	58	35	.....	100	tr	68	88	29	4	96	85	14	40	31	30	28
3708A–4	Reisolation of 3257A–2	Carrot-red	60	40	.....	54	.....	82	tr	25	24	tr	1	8	45	tr	tr	9	40	20

[a] Canada bluegrass.
[b] *Bromus rigidus.*

The results obtained indicate that both the pink and carrot-red strains are sometimes capable of causing seed rot but that the species is seldom actively parasitic. Isolates frequently were obtained from plants growing in nitrogen-starved soil, in crowded volunteer stands, or from plants growing in cool wet soil. The fungus apparently is a competitor with the plant roots for soil nutrients and its action in general is probably detrimental. The fungus needs study to determine its straw-rotting ability and to determine if its injury is possibly the result of enzymatic action.

MORPHOLOGY

Conidia of both the pink and carrot-red strains appear to be identical (Fig. 1) although spores of the pink form tend to "blow up" to a larger size in old, semi-vacuolated spores. Younger but mature spores of both types are hyaline and non-guttulate but they soon become 1–4 guttulate and somewhat distended. The spores average 4–9 × 1.7–3.0 μ but some

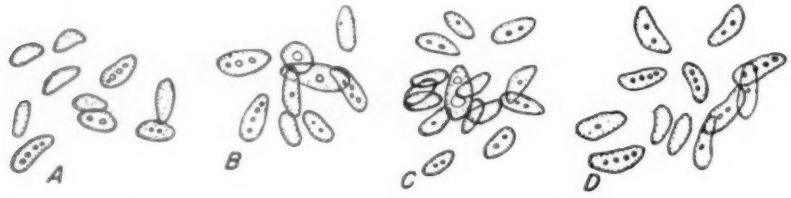

FIG. 1. Conidia of *Gloeosporium bolleyi* from pure cultures on potato-dextrose agar. *A*. from culture 3679 B–5, pink strain, isolated from *Hordeum brevisubulatum*, under snow, Mandan, N. Dak., Jan. 17, 1946. *B*. from culture 49 A–2, pink strain, isolated from *Agropyron cristatum*, Huron, South Dakota. *C*. from culture 5 B–1, intermediate strain, from *A. cristatum*, Mandan, N. Dak. *D*. from culture 3703 B–3, carrot-red strain, from *Elymus canadensis*, Mandan, N. Dak. (All × 1000.)

are as wide as 4 μ; and extremely swollen or distorted ones average considerably larger.

Scattered among some of the colonies of conidia are chlorinous chlamydospores with the inner wall (endospore) somewhat reticulate. The hyphae formed from germinating spores are variable but usually are compacted to a carbonaceous, almost amorphous mass in older cultures. Bodies formed at the edge of older cultures appear to be stromatic.

TAXONOMY

Many workers have seen this *Gloeosporium* on Gramineae but it has frequently been confused with *Colletotrichum graminicola* (Ces.) G. W. Wils. The spores of the latter as given by Manns (*C. cereale* Manns) (6) are spindle-form to boat shaped, 18–26 × 3–4 μ. In addition *C. graminicola*, according to Manns, produced steel gray to dark gray, compact downy growth and sporulation was not plentiful. The *Gloeosporium* under study has spores 4–9 × 1.7–4.0 μ and typically produces primary mucose colonies very different from those of *C. graminicola*. Because of the absence of setae, it belongs in the genus *Gloeosporium*.

Colletotrichum graminicola was reported on cereals in North Dakota by Bolley (1) but an illustration (by Milbrath, Fig. 21, *a*) in that article indicates that the North Dakota workers were probably dealing, in part at least, with the same *Gloeosporium sp.* that is the subject of this article. Böning and Wallner (2) describe a seedling blight, wilt, and footrot due to *C. graminicola* in Germany, while Sanford (5) found that this species caused a root rot in oats near Edmonton, Alberta. Winter (9) found the same type of fungus on wheat, barley, rye, and oats in Germany. All of these workers appear to have been dealing with the true *Colletotrichum* and not *Gloeosporium*.

There are two species of *Gloeosporium* described on Gramineae, both by Rostrup from Denmark (4). *G. graminum* on *Lolium multiflorum* has spores 11–14 × 4–6 μ while *G. dactylidis* on *Dactylis glomerata* has small spores, 5 × 1 μ. Neither is similar to our fungus, which appears to be undescribed in spite of its prevalence. Since the most obvious name (*graminum*) has been used and since the host range and geographical distribution are so extensive, it appears desirable to name this species for an individual. We therefore name this for Henry L. Bolley, who noted the fungus early in the rootrot work and who pioneered in this subject. The fungus is described as follows:

Gloeosporium bolleyi sp. nov.

Acervulis (pseudopionnotes) in cultura copiosis, mucosis primo incarnatis v. aurantio-rubris demum nigris, carbonaceis; conidiis numerosis in cultura, hyalinis, subphaseoliformibus v. oblongis, eguttulatis v. demum guttalatis, 4–9 × 1.7–4.0 μ, plerumque 5–8 × 1.9–2.5 μ. Hab. in rhizoideis languidis Graminearum, North Dakota, South Dakota, Nebraska, Wyoming, Montana, Minnesota et Washington. Typus est cultura 4180B–4, ex *Tritico aestivo*, Mandan, North Dakota.

The description is based on pure cultures of the fungus. The culture used as the type is a typical pink strain isolated from common wheat in the rotation plots at the Northern Great Plains Field Station, Mandan, N. Dak. Dried sub-cultures of the type have been preserved and filed in several herbaria.

SUMMARY

Gloeosporium bolleyi Sprague is common on the roots of many species of grasses and cereals in the western United States. Under artificial inoculation conditions in the greenhouse it sometimes causes seed rot and root necrosis of small grains and grasses.

The writer is again indebted to Miss Edith Cash for checking the Latin description and to Dr. A. G. Johnson for editing the manuscript.

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PHYSIOLOGICAL EXHAUSTION OF STRAWBERRY PLANTS AS A FACTOR IN WINTER KILLING

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(Accepted for publication October 28, 1947)

Mulching strawberry plants after they have been subject to "hardening" temperatures has reduced winter killing in Minnesota. Plants were injured when hardening temperature fell below – 6° C. and were killed at temperatures of – 12° C. and lower (2). Freezing and thawing temperatures, especially those ranging from – 6° C. to 6° C., occur during the fall and spring when strawberry plants are not mulched and occur frequently under the ice mulch cover during winter and spring. The question arises as to whether such fluctuating temperatures bring about physiological changes in the plants to make them more subject to winter injury or to predispose them to soil inhabiting pathogens.

EXPERIMENTAL MATERIALS AND METHODS

Field-grown, current-season plants of the variety Senator Dunlap obtained from Allens Nursery, Salisbury, Md., were used. These plants were obtained on two different dates, October 21 (Series 1) and November 15, 1946 (Series 2). The root systems for plants of both series were well developed, but plants of series 2 had more leaves and more fully formed crowns than plants of series 1.

Although the plants were free of disease so far as could be ascertained, undoubtedly some of them had incipient infections by root-rotting fungi. They also carried with them on their roots several organisms not necessarily pathogenic to them. In order to assure the presence of soil inhabiting pathogens, all plants of series 1 and part of those of series 2 were inoculated with isolates of *Fusarium* pathogenic to strawberry plants. The Fusarium isolates were grown on a sand-cornmeal medium. The plant roots were immersed in a suspension of these fungus cultures and were wrapped in moist sphagnum moss prior to storage. The plants were examined periodically throughout the storage period and the sphagnum wrappers were kept moist. Plants were stored at a constant temperature (3° C.) or at a fluctuating temperature (3° C. alternating with – 3° C.) for 12, 14, or 24 days (Table 1).

After each respective storage period, five plants were selected at random for analyses of total sugars (reducing and non-reducing) and starch. Triplicate determinations were made on each group from 2-gram, dry-weight samples. The Hagedorn-Jenson method for sugar determination was used (3). The remaining plants were potted in sterilized soil and placed in a greenhouse at 16.5° C., where they had 14 hours of light per day to insure the longday effect on growth.

[1] Paper No. 2344, Scientific Journal Series, Minnesota Agricultural Experiment Station, 1947.

TABLE 1.—*Storage conditions for strawberry plants*

Series and lot	Number of days in storage at									Total number of days in storage
	3° C.	– 3° C.	3° C.	– 3° C.	3° C.	– 3° C.	3° C.	– 3° C.	3° C	
1, A	14	.....	.....	.....	.....	.....	.....	.....	.....	14
1, B	4	6	4	.....	.....	.....	.....	.....	.....	14
2, A	12	.....	.....	.....	.....	.....	.....	.....	.....	12
2, B	24	.....	.....	.....	.....	.....	.....	.....	.....	24
2, C	.....	3	3	4	2	.....	.....	.....	.....	12
2, D	.....	3	3	4	2	3	3	3	3	24

ANALYSES, RESULT, AND DISCUSSION

There was more sugar in the roots and tops of plants subjected to alternate freezing and thawing during 12 and 14 days of storage than in plants stored for the same times at a constant temperature of 3° C. (Table 1). After 24 days in storage, sugars had increased slightly in the roots, but decreased slightly in the tops of plants held at a constant temperature of 3° C.; but the sugars in the entire plants were approximately the same as they had been after 12 days in storage (Table 2, series 2, B and A). At fluctuating temperatures there was a lower sugar content in both roots and tops of plants stored for twenty-four days than in plants stored for twelve days (Table 2, series 2, D and C).

Starch content decreased very rapidly in the roots and rather slowly

TABLE 2.—*Sugar and starch determinations for strawberry plants stored at a constant temperature or at fluctuating temperatures*

Determinations	Stored at 3° C.		Stored at 3° C. alternating with – 3° C.	
	Milligrams of sugar or starch in one gram dry tissue			
	Roots	Tops	Roots	Tops
	Series 1, A[a]		*Series 1, B*[a]	
Total sugars	60.0	30.0	70.0	43.0
Reducing sugar	50.7	25.0	55.0	36.3
Nonreducing sugar	9.3	5.0	15.0	6.7
Starch as glucose	22.5	31.3	17.5	27.5
	Series 2, A		*Series 2, C*	
Total sugars	98.0	40.0	115.0	48.0
Reducing sugar	76.0	40.0	91.5	41.3
Nonreducing sugar	22.0	0.0	23.5	6.7
Starch as glucose	45.0	50.0	40.0	45.0
	Series 2, B		*Series 2, D*	
Total sugars	102.5	35.3	98.0	30.0
Reducing sugar	78.5	35.3	76.5	29.0
Nonreducing sugar	24.0	0.0	21.5	1.0
Starch as glucose	25.0	48.1	21.9	37.5

[a] Series 1, A and B were stored for 14 days; Series 2, A and C for 12 days; Series 2, B and D for 24 days.

in the tops as the storage period lengthened. There was less starch in the plants stored at fluctuating temperatures than in those held at constant temperatures (Table 2).

Plants of series 2, which had a relatively vigorous crown development when placed in storage, contained more total sugar and starch in their roots and tops after twenty-four days of storage than those of series 1 that were stored only fourteen days.

New roots and shoots were observed on plants removed from storage and placed in the greenhouse for twelve days. They appeared on a higher percentage of plants stored at fluctuating temperatures than on plants stored at a constant temperature, regardless of the length of the storage (Table 3). Cold treatment apparently had a stimulatory effect on the plants, and re-

TABLE 3.—*Growth responses of strawberry plants in the greenhouse after different storage temperature treatments*

Series and storage condition	Total number of plants[a]	Percentage of plants in each growth response class after							
		12 days				36 days			
		Dead	Inactive	Weak	Vigorous	Dead	Inactive	Weak	Vigorous
Series 1 (14-day storage)									
A. 3° C.	30, Inoc.	0	60	40	0	17	0	40	43
B. 3° C. alternating with −3° C.	30, Inoc.	10	33	57	0	60	0	33	7
Series 2 (12-day storage)									
A. 3° C.	10	0	90	10	0	0	0	20	80
A. 3° C.	20, Inoc.	0	70	30	0	10	0	20	70
C. 3° C. alternating with −3° C.	10	0	30	60	10	30	0	40	30
C. 3° C. alternating with −3° C.	20, Inoc.	5	20	75	0	40	0	35	25
Series 2 (24-day storage)									
B. 3° C.	10	0	80	20	0	10	0	20	70
B. 3° C.	20, Inoc.	0	60	40	0	15	0	20	65
D. 3° C. alternating with −3° C.	10	0	30	70	0	50	0	40	10
D. 3° C. alternating with −3° C.	20, Inoc.	10	15	75	0	60	0	30	10

[a] Inoc. signifies that the plants were inoculated with Fusarium isolates.

sulted in an earlier growth response. This growth response did not result in more vigorous plants, because, after thirty-six days in the greenhouse, vigorous plants were more numerous in the lots stored at constant temperature. The higher sugar content in roots and tops of the plants stored at the alternating temperatures can be considered responsible for the earlier or quicker growth response. The depleted carbohydrate reserve however was not sufficient for subsequent growth and establishment of the plants, hence, many of them remained weak or died.

A similar growth response was observed after cold treatment with chrysanthemum cuttings by Mader and Phillips.[2] These workers subjected

[2] Mader, E. O., and Kyle Phillips. Induced root formation of chrysanthemum cutting by low temperatures. Not published.

chrysanthemum cuttings to temperatures ranging from 0.5 to 1.5° C. and found that such an exposure (6–21 days depending on varieties used) was equal in its effect to the treatment of cuttings with 50 p.p.m. indole butyric acid solution. Furthermore, such cuttings treated at low temperature (and also cuttings treated with indole butyric acid) contained a higher percentage of total sugars and less starch than nontreated cuttings. For any given variety used there was an optimum range within the low temperature treatment that gave not only an earlier rooting but also allowed for a normal development of the tops. When this range was overstepped, the time necessary for rooting was decreased but at the expense of shoot or top development, so that either a weak plant or death often resulted.

Strawberry plants stored at constant temperature (3° C.) survived better than those stored at fluctuating temperatures (3° C. alternating with −3° C.). Sixty per cent of the plants in series 1, B, were dead 36 days after removal from storage at fluctuating temperature as compared with 17 per cent in series 1, A which had been stored at constant temperature (Table 3). Comparable losses occurred in series 2, but because plants of series 2 had greater vigor than those of series 1 before storage, the losses did not reach 60 per cent and 17 per cent, respectively, unless the storage period was 24 days.

Inoculation with isolates of *Fusarium* known to be pathogenic to strawberries appeared to increase losses only slightly (Table 2). Approximately 10 per cent more of the inoculated plants died than noninoculated plants. To what extent these losses are due to a weakening of the plants which results in physiological exhaustion and finally death, or due to the action of soil-inhabiting pathogens, or to the interaction of these factors remains to be proven.

Similar results were obtained by Eide and Voorhees,[3] who subjected inoculated and noninoculated plants (inoculated with isolates of *Fusarium* pathogenic to strawberries) to temperatures of −3° C. There were numerous lesions on the roots of inoculated plants and they wilted after they were removed from the low temperatures and placed in the greenhouse. The noninoculated plants also subjected to freezing treatments did not differ greatly from the noninoculated plants left in the greenhouse for comparison. Plants inoculated but not subjected to freezing had few lesions on the roots and there was no loss of vigor. These results are in agreement with those found by the present writers, except that in our experiments noninoculated plants subjected to alternate freezing and thawing were greatly impaired in vigor. It is possible that presence of incipient infections or activity of saprophytes on the roots of these field grown plants may account for this difference.

It is a known fact that when temperatures are below 4.5° C., carbohydrates are transformed in many plants (5, 6). This is followed by marked acceleration in respiration, particularly when temperatures drop to about

[3] Eide, Carl J., and R. K. Voorhees. Unpublished data. University of Minnesota.

0° C. (1, 4). Under the conditions of these tests carbohydrate conversion and apparent increased respiration took place in the strawberry plants. This conversion was also apparent in the plants stored at the constant temperature of 3° C. (a storage temperature of 5° C. was not available during the time these were run) but was more pronounced in those kept at the alternating temperatures during storage. The decrease of starch and in particular the decrease of sugar in the plants held for 24 days at the alternating temperatures suggest that increased respiration under these conditions must have been responsible for the physiological exhaustion and subsequent death of the plants.

These experiments indicate that strawberry plants subjected to alternating freezing and thawing temperatures will undergo a physiological weakening or exhaustion leading to ultimate death. Soil inhabiting plant pathogens are not necessary to bring about the death of such plants, but their presence will be a contributing factor in the killing of the plants. It appears that whatever means and methods are taken to maintain the carbohydrate reserve in the strawberry plants will reduce the probability of losses due to winter-killing.

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THE CARBOHYDRATE METABOLISM OF GERMINATING PHYMATOTRICHUM SCLEROTIA WITH SPECIAL REFERENCE TO GLYCOGEN[1]

DAVID R. ERGLE[2]

(Accepted for publication November 3, 1947)

Previous studies (3, 4) of the root rot fungus, *Phymatotrichum omnivorum,* provided the basis for the work here reported. In those studies it was observed that sclerotia, formed in soil cultures of the fungus, continued to accumulate substantial amounts of polysaccharides during a 65-day developmental period. The major portion of the polysaccharides was identified as free and bound glycogen which together comprised approximately 37 per cent of the dry-weight of mature sclerotia.

Conceivably, these reserves play a vital role in the perpetuation of the fungus which, in the sclerotial stage, is known to have remained viable for several years (8). It follows that information on the mechanism of mobilization and utilization of these reserves would be of interest as a contribution to the basic knowledge of the fungus. The obtaining of such information was the primary objective in the current investigation.

MATERIALS AND METHODS

Production, Germination, and Preparation of Sclerotia for Analysis

Using navy beans as the principal organic nutrient, sclerotia were produced by culturing *Phymatotrichum omnivorum* in Houston Black clay (2). These were separated from the soil by careful washing and screening, and the surface water was removed by blotting prior to weighing. Sclerotia thus obtained from three lots of 30- and 60-day-old cultures of *P. omnivorum* were used separately in a series of germination experiments. Samples from each of the lots were retained for chemical analysis so as to establish the composition of sclerotia at the start of each experiment.

Three-gram portions of sclerotia were weighed into sterile Petri dishes and surface sterilized by agitating with a 0.5 per cent solution of sodium hypochlorite for one minute. The solution was decanted and the sclerotia were washed once with sterile tap water and again with a sterile, dilute inorganic nutrient solution (7) pH 6.5. About 2 ml. of the latter was left to cover the bottom of the dishes without submerging the sclerotia. The Petri dishes, 50 for each of the lots of 30- and 60-day sclerotia, were placed in an incubator maintained at 28° –30° C., and the sclerotia were allowed to germinate and the mycelium to develop for periods of 3, 6, 9, and 12 days. On the 6th day an additional 2 ml. of the nutrient solution was added to replace water losses and to maintain the pH at or near 6.5.

[1] Published with the approval of the Director of the Texas Agricultural Experiment Station as Technical Paper No. 1056.

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At the end of each of the allotted periods of incubation the sclerotia and mycelium of 6 Petri dishes were combined into a single sample, killed in toluene vapor, and dried in a mechanical convection oven at 70° C. for 18 hours. After weighing for the determination of the dry weight, the material was finely ground in a semi-micro Wiley mill preparatory to analysis. The contents of 4 additional Petri dishes, two for each analysis, were used for the determination of amylolytic activity.

Chemical Methods

Amylolytic Activity.—The determination was made according to Spoehr's (6) procedure in which the increase in the reducing power of the reaction mixture (2 gm. soluble starch, 5 ml. phosphate solution, fresh sclerotia and mycelium from 2 Petri dishes, and water to make 250 ml.) was measured after incubating for 24 hours at 30° C. The results were expressed as milligrams of glucose per gram of oven-dry tissue.

Sugars.—The sugars were extracted with boiling 80 per cent ethanol in a Soxhlet unit and the reducing sugars were determined by the method of Wildman and Hansen (11). Nonreducing sugars (4) were determined after hydrolysis with 4 per cent hydrochloric acid (by weight) for 1.25 hours in an autoclave at 15 lb. pressure.

Mannitol.—The presence of mannitol in the sclerotia and mycelium was established by isolation in a pure form from a 95 per cent ethanol extract and the preparation of the hexaacetyl derivative, the melting point of which agreed with that recorded in the literature (5). Using the method of Todd *et al.* (10) mannitol was quantitatively determined in an aliquot of the aqueous solution used for the determination of sugars.

Free Glycogen.—The oven-dried, insoluble residue from the extraction of sugars was weighed and the free glycogen was extracted from duplicate 0.5-gm. samples with 10-ml. portions of hot water until a test for glycogen (a reddish brown color with dilute iodine solution) in the extract was negative. To the combined extracts of each sample containing 5 ml. of a citrate buffer solution at pH 5.0, there was added 0.2 gm. Takadiastase,[3] a few drops of toluene to insure sterility, and water to make a total of 50 ml. The digests, contained in tightly stoppered flasks, were incubated at 32° C. for 48 hours. The reducing sugars resulting from hydrolysis were determined and multiplied by 0.9 to convert to the glycogen equivalent.

Bound Glycogen.—The wet residue from one of the duplicate samples extracted for free glycogen was suspended in 5 ml. of 35 per cent aqueous potassium hydroxide and heated for 1 hr. at 15 lb. pressure. After removal of the insoluble cell-wall materials by centrifugation, the glycogen was precipitated from the cold alkaline solution by the addition of 3 volumes of 95 per cent ethanol and acidulation with concentrated hydrochloric acid. The precipitated glycogen was next washed with ethanol, dried, dissolved in water, and determined as in the case of free glycogen.

[3] The Takadiastase was a special, undiluted preparation of Parke, Davis, and Co.

Hemicellulose.—The remaining duplicate from the extraction of free glycogen was hydrolyzed with 4 per cent hydrochloric acid and the resulting sugars were determined and multiplied by the factor 0.9. The value thus obtained represented the sum of (a) bound glycogen and (b) hemicellulose. The latter was calculated by subtracting the value of (a) from that of (a) + (b).

Cellulose and Suberin.—These constituents were separately determined on hemicellulose-free samples of sclerotia and mycelium by methods described in detail elsewhere (4).

Total Carbohydrates.—Calculated as the sum of the determined carbohydrate fractions.

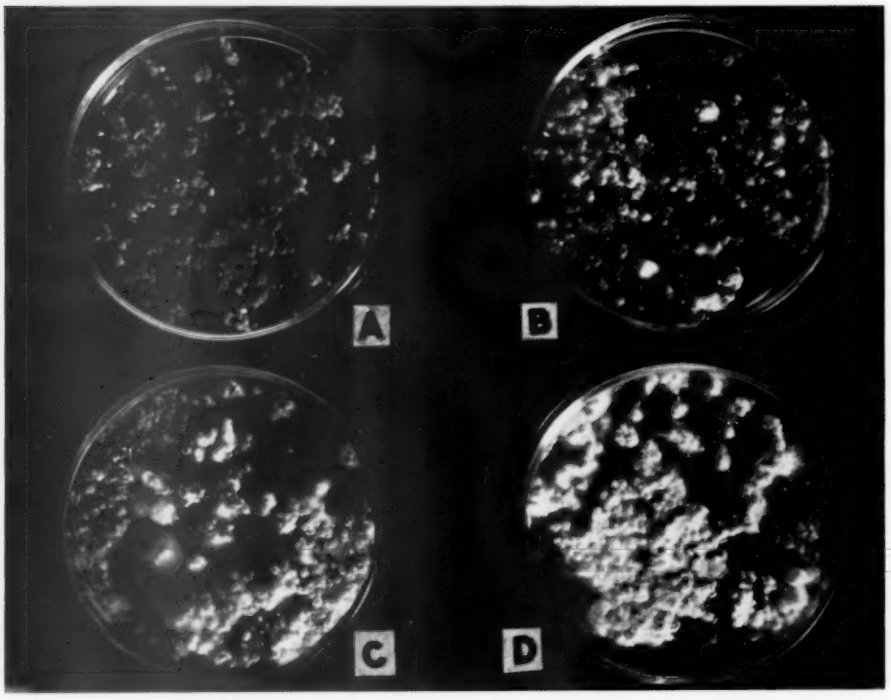

FIG. 1. Dormant sclerotia (A), and those showing mycelial growth after incubating for periods of 3 (B), 6 (C), and 9 (D) days.

The chemical data are reported (a) as percentage of dry weight after the respective periods of germination and (b) as grams of constituents in the sclerotia and mycelium resulting from the initial 18 grams of fresh sclerotia. Each value is the average obtained from germination experiments that were replicated three times at three different dates.

RESULTS

Germination

Approximately 48 hours from the start of incubation, the initiation of germination processes was evidenced by the emergence of small, white tufts

of mycelium from the sclerotia. As illustrated in figure 1 the density of mycelial growth increased with time except that there was no visible difference after 9 days. Respiration, as measured by loss in dry weight, was high during the initial stage of germination, but after the 9th day there was little detectable change. The two lots of sclerotia, though differing in age and in chemical composition, were remarkably similar in this respect. The 12-day losses in dry matter by 30- and 60-day sclerotia were 16.79 and 16.40 per cent of their original dry weights respectively, of which more than half of these losses occurred during the first 3 days of germination.

Changes in Composition of Germinating Sclerotia

The changes in the composition of germinating sclerotia as a percentage of the successive dry weights are given in table 1. The behavior of the

TABLE 1.—*Chemical changes during the germination of sclerotia and growth of mycelium. Data reported as percentage of dry weight*

Constituent	Sclerotia before germination	Sclerotia plus mycelium after germination			
		Days			
		3	6	9	12
	Sclerotia from 30-day-old cultures				
Mannitol	5.42	7.11	7.61	7.89	8.46
Reducing sugars	0.14	1.24	2.60	3.15	2.86
Nonreducing sugars	1.62	1.37	0.00	0.00	0.00
Free glycogen	10.57	2.64	1.94	1.32	2.56
Bound glycogen	26.35	26.71	20.21	18.41	17.83
Hemicellulose	9.90	10.40	11.46	12.27	10.81
Cellulose	0.60	0.68	0.65	0.91	0.90
Total carbohydrates	54.60	50.15	44.47	43.95	43.42
Suberin	2.88	3.84	4.69	4.32	5.01
	Sclerotia from 60-day-old cultures				
Mannitol	3.90	4.85	4.74	5.04	4.44
Reducing sugars	0.80	0.52	0.02	0.94	2.00
Nonreducing sugars	0.73	0.25	0.26	0.00	0.00
Free glycogen	4.07	2.03	1.67	1.79	1.31
Bound glycogen	30.85	24.34	21.82	17.94	16.39
Hemicellulose	12.23	16.02	16.63	16.37	14.83
Cellulose	0.68	0.86	0.88	1.05	1.12
Total carbohydrates	53.26	48.87	46.02	43.13	40.09
Suberin	2.74	3.81	4.87	5.31	5.20

concentrations of water-soluble and water-insoluble carbohydrate constituents as germination and mycelial development progressed is shown in figure 2. The actual amounts of the various carbohydrate fractions involved in the germination are given in table 2 as grams of constituents resulting from the initial 18 grams of fresh sclerotia.

The germination of 30-day sclerotia for a period of 12 days resulted in actual losses of nonreducing sugars, free glycogen, bound glycogen, and hemicellulose (Table 2). Gains were found in mannitol, reducing

sugars, cellulose, and suberin. The results for 60-day sclerotia differed in the following respects: (a) there was a slight loss in mannitol and (b) a gain in hemicellulose. Considering the net changes in all of the carbohydrate fractions as a whole, *i.e.*, total carbohydrates, it was found that 18 grams of sclerotia from 30- and 60-day-old cultures decreased in carbohydrate content by 1.22 and 1.24 grams, respectively.

The initial 3-day period was characterized by relatively large losses in the free and bound glycogen contents of sclerotia. During this time the

TABLE 2.—*Chemical changes during the germination of sclerotia and growth of mycelium. Data reported as grams of constituents per initial 18 grams of fresh sclerotia*

Constituent	Sclerotia before germination	Sclerotia plus mycelium after germination				Gain or loss during germination
		Days				
		3	6	9	12	
	Sclerotia from 30-day-old cultures					
Total dry weight	6.55	6.00	5.64	5.53	5.46	−1.09
Mannitol	0.36	0.43	0.43	0.44	0.46	+0.10
Reducing sugars	0.01	0.07	0.15	0.17	0.16	+0.15
Nonreducing sugars	0.11	0.08	0.00	0.00	0.00	−0.11
Free glycogen	0.69	0.16	0.11	0.07	0.14	−0.55
Bound glycogen	1.73	1.60	1.14	1.02	0.97	−0.76
Hemicellulose	0.65	0.62	0.65	0.68	0.59	−0.06
Cellulose	0.04	0.04	0.04	0.05	0.05	+0.01
Total carbohydrates	3.59	3.00	2.52	2.43	2.37	−1.22
Suberin	0.19	0.23	0.26	0.24	0.27	+0.08
Amylolytic activity[a]	53	60	71	82	95	+ 42
	Sclerotia from 60-day-old cultures					
Total dry weight	6.28	5.73	5.49	5.36	5.25	−1.03
Mannitol	0.24	0.28	0.26	0.27	0,23	−0.01
Reducing sugars	0.05	0.03	Trace	0.05	0.11	+0.06
Nonreducing sugars	0.05	0.01	0.01	0.00	0.00	−0.05
Free glycogen	0.26	0.12	0.09	0.10	0.07	−0.19
Bound glycogen	1.94	1.40	1.20	0.96	0.86	−1.08
Hemicellulose	0.77	0.92	0.91	0.88	0.78	+0.01
Cellulose	0.04	0.05	0.05	0.06	0.06	+0.02
Total carbohydrates	3.35	2.81	2.52	2.32	2.11	−1.24
Suberin	0.17	0.22	0.27	0.28	0.27	+0.10
Amylolytic activity[a]	80	147	183	198	217	+ 137

[a] Mgs. of glucose per gm. of dry material.

free glycogen content of germinating 30- and 60-day sclerotia decreased 77 and 50 per cent, respectively, of the amounts originally present; the bound glycogen content of the 30-day sclerotia remained practically unchanged; and that of the 60-day sclerotia decreased 28 per cent. The remaining 9 days of germination and mycelial growth resulted in further progressive decreases in both free and bound glycogen except that the free glycogen content of germinating 30-day sclerotia was increasing by the 12th day.

The nonreducing and reducing sugars were present in ungerminated

sclerotia in relatively small amounts, but the latter in germinating 30-day sclerotia tended to increase linearly with time while the nonreducing sugars disappeared by the 6th (30-day sclerotia) and 9th days (60-day sclerotia) and were not reformed during the remaining period of the experiment. In the germination of the 60-day sclerotia the reducing sugar content approached zero in the first 6 days but subsequently increased to show a net gain of100 per cent by the end of the experiment.

The mannitol content of both lots of sclerotia before and during germination exceeded by far that of the true sugars. Whereas the quantity of mannitol in germinating 30-day sclerotia increased consistently with time

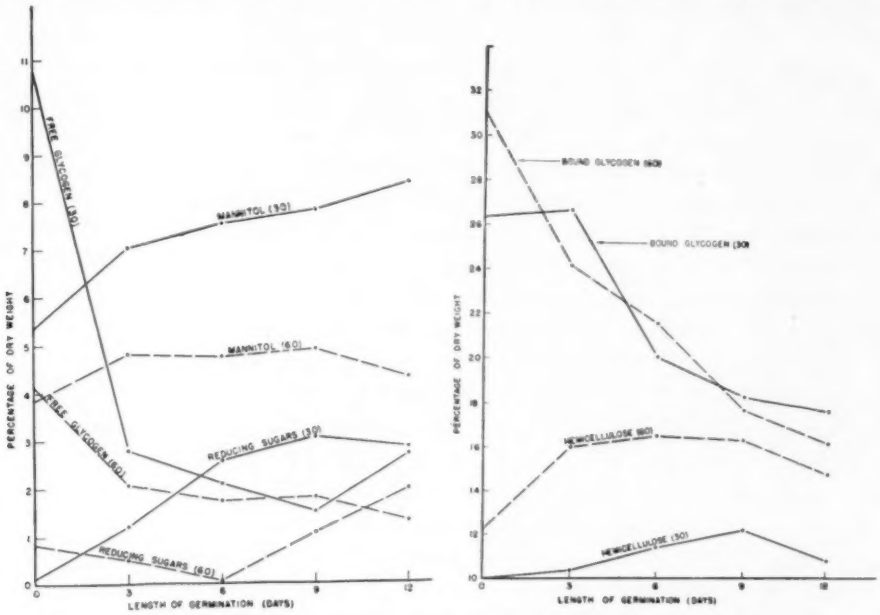

FIG. 2. Left. Variations in concentration of the soluble carbohydrate fractions of germinating 30- and 60-day sclerotia.

Right. Variations in concentration of the insoluble carbohydrate fractions of germinating 30- and 60-day sclerotia.

and showed a net gain of 28 per cent, increases in the 60-day sclerotia were limited to a 9-day period after which there was a net loss of 4 per cent.

By the 9th day the quantity of hemicellulose in germinating 30 and 60-day sclerotia had increased 5 per cent and 14 per cent, respectively, but these increases were followed by decreases large enough to show a net loss of approximately 10 per cent in the former and a net gain of only 1 per cent in the latter. Cellulose and suberin were present in both ages of ungerminated sclerotia in approximately equal amounts, which increased 25 per cent and 42 per cent, respectively, during the germination of 30-day sclerotia, and 50 per cent and 59 per cent, respectively, in the 60-day sclerotia.

Amylolytic Activity

Amylolytic activity (Table 2) in the germinating sclerotia increased with time. The results on 30-day sclerotia showed a fairly constant rate of increase while those from 60-day sclerotia showed a diminution in the rate of increase. A more striking difference in both lots of sclerotia was found to be in their comparative amylolytic activities before and during germination. Before germination the ratio value of the activity in 60-day sclerotia to that in 30-day sclerotia was 1.6, but after germination started it increased to 2.4 and remained practically constant throughout the experiment.

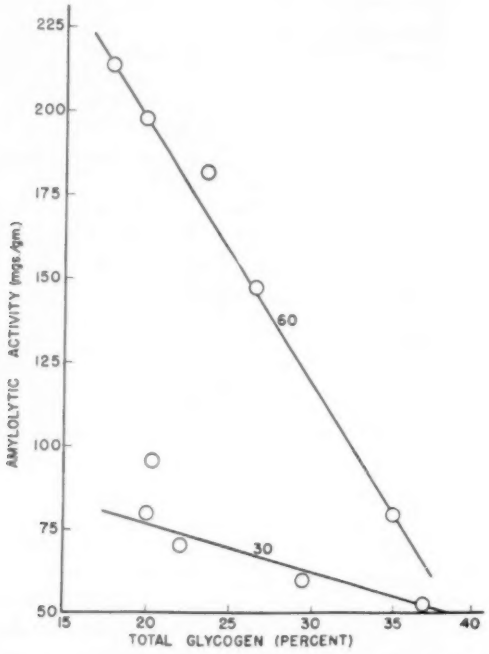

FIG. 3. Relation of total glycogen and amylolytic activity in germinating 30- and 60-day sclerotia.

Amylolytic activity in the germinating 60-day sclerotia was found to vary inversely with the concentration of total glycogen (free + bound), and a similar relation, though less consistent, was indicated during the germination of 30-day sclerotia (Fig. 3). In addition it was noted that a higher level of amylolytic activity (60-day sclerotia) was associated with higher levels of the insoluble carbohydrate metabolites.

DISCUSSION

The chemical and physiological processes found occurring in germinating sclerotia seem to reflect a pattern of metabolic sequences that is perhaps somewhat similar to that in germinating starchy seed. Like the latter, sclerotia of *Phymatotrichum omnivorum* are deficient in sugars and

the processes of respiration and synthesis depend upon the mobilization of sugars from other more abundant carbohydrates. The results of this study clearly show that glycogen, both free and bound, was the principal source of carbohydrate substrates with indications that nonreducing sugars and hemicellulose were sources of minor amounts. The mode of glycogen hydrolysis to glucose or to other metabolizable forms is suggested by the observed two-fold increase in amylolytic activity after germination started. The possibility that additional enzymes, glycogenase for example, were also functioning in the hydrolysis of glycogen is very likely. Also, Blank and Talley (1) showed that the mycelium contains additional carbohydrases that are capable of hydrolyzing a variety of simple and complex carbohydrates.

As reflected by the data, the next phase in the metabolic pattern was the utilization of the sugars mobilized from the various sources mentioned. Assuming that carbohydrates alone were used, the data show that respiration alone probably accounted for 89 per cent (30-day sclerotia) to 83 per cent (60-day sclerotia) of the observed losses. It is unlikely that either the fat (2.17 per cent of dry weight) or protein (18.98 per cent of dry weight) reserves (4) of sclerotia contributed materially to respiration as they are present in much smaller amounts than the carbohydrates. The remaining loss in carbohydrates may be logically attributed to the synthesis of noncarbohydrate constituents, such as suberin, by the mycelium. The utilization of some of the mobilized carbohydrates in the synthesis of mannitol, hemicellulose, and cellulose also was indicated as these constituents accumulated either throughout or during the greater part of the 12-day study. The accumulation of cellulose and suberin may be ascribed to cell wall formation by the mycelium of germinating sclerotia.

The results as a whole show that differences in composition and in age of sclerotia did not materially influence either the general course or the rate of carbohydrate metabolism. Before germination the 30-day sclerotia contained approximately twice the soluble carbohydrate (mannitol + sugars + free glycogen) content of the 60-day sclerotia while the latter were appreciably richer in insoluble reserves (bound glycogen + hemicellulose). Nevertheless carbohydrate utilization in the two lots of germinating sclerotia seemed to proceed at about equal rates, using the losses in dry matter and in carbohydrates as criteria. In this connection the finding of a higher amylolytic activity to be associated with higher levels of insoluble carbohydrate reserves might have been a factor in the equalization of the metabolic rates.

Since this study has shown that the glycogen reserves of sclerotia are the chief carbon sources during germination, the observed termination of mycelial growth after 9 days poses the question of possible glycogen depletion as a factor in limiting further growth. The data on the metabolism of glycogen are of little value in this respect as they reflect not only the

unused glycogen in the sclerotia residues but also that transported to or resynthesized in the mycelium. However, evidence of the presence of adequate amounts of glycogen in the sclerotia residues for further growth was obtained when it was found that the 12-day residues could be germinated a second time after the previous mycelial growth had been removed. In other laboratory studies Taubenhaus and Ezekiel (9) also showed that Phymatotrichum sclerotia under certain conditions may be germinated as many as five times. Thus it is apparent that the glycogen reserves of sclerotia are not readily exhausted during germination, and that the cessation in mycelial growth found in this study must be attributed to other causes.

SUMMARY

Sclerotia from 30- and 60-day-old soil cultures of *Phymatotrichum omnivorum* were allowed to germinate and grow on a sterile, inorganic nutrient substrate for periods of 3, 6, 9, and 12 days and the metabolism of their carbohydrate reserves was studied. The principal results may be summarized as follows:

1. The course and the rate of carbohydrate metabolism in the two ages of germinating sclerotia were essentially the same notwithstanding certain differences in the initial composition. Germination of 18 grams each of 30- and 60-day sclerotia resulted in losses of 1.09 and 1.03 grams of dry matter, respectively, and in corresponding losses of 1.22 and 1.24 grams of carbohydrates.

2. The initial 3-day period was characterized by intense metabolic activity as reflected in the loss during that time of approximately half of the total amount of carbohydrates used in the 12-day experiment.

3. Germination resulted in substantial net losses of nonreducing sugars, free and bound glycogen, and some hemicellulose (the latter during the 9–12 day period). Reducing sugars, mannitol (0–9 day period), cellulose, and suberin showed increases.

4. The glycogen reserves (free and bound) of sclerotia were the chief source of substrates for respiration and synthesis. The former process accounted for 83–89 per cent of the observed loss in total carbohydrates.

5. Glycogen mobilization was associated with a relatively high level of amylolytic activity, which tended to vary inversely to the concentration of total glycogen in the germinating sclerotia.

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PHYTOPATHOLOGICAL NOTES

Rootstock Susceptibility to Armillaria mellea.—The Santa Clara Valley is one of the principal deciduous fruit areas of California, devoted mainly to apricots, pears, and prunes. Pears on French pear roots are sufficiently resistant to Armillaria root rot to be excluded from this report. It may be mentioned, however, in passing, that pear trees on quince roots may be killed by this fungus. Apricot and prune orchards, on the other hand, have already sustained a loss of more than 10 per cent of the trees according to a survey[1] of 65 properties selected at random.

The most effective chemical treatment of the soil thus far, the injection of carbon disulfide, has been, on the whole, disappointing in this area. Unpublished work of the first and last authors indicates that this is partly due to failure of the chemical to penetrate to the lower depths (5 to 10 feet) in the rather heavy moist soils.

The alternative most commonly resorted to in Armillaria-infested areas of this valley is the planting of Persian walnut (*Juglans regia*) on Northern California Black walnut (*J. hindsii*) roots which seem to be adequately resistant to *Armillaria*. However, the disorder known as black line of walnut[2] is sufficiently prevalent in the area to raise some doubt as to the success of such plantings.

It seems to be obvious, at any rate, that an Armillaria-resistant rootstock for apricot and prune is needed in the Santa Clara Valley.

Eighteen lots of plum roots, mostly Myrobalan and Marianna types, were planted for trial at the University of California Deciduous Fruit Field Station at San Jose in 1928. These lots contained 37 to 40 trees and were planted about 36 inches apart in rows 48 inches apart. This crowded planting eventually necessitated pruning off all the lower branches to facilitate cultivation. Moreover, approximately half of each row was cut back heavily in the 2nd and 4th years from planting to simulate the usual procedure in topworking such trees in orchards. All of the trees were inoculated repeatedly by placing a handful of wood containing the fungus from pure culture adjacent to the crown. All the circumstances seem to have combined to provide a severe test of resistance. However, few of the trees were killed by *Armillaria* during the first five years. Trees which died were examined as soon afterward as feasible to determine the cause of death. Only those which seemed clearly to have been killed by *Armillaria* are so indicated in the summary of Table 1. The surviving trees were pulled in 1945, and the degree of root injury rated on a scale of 0 to 10, in which Class 0 was to represent trees with no infection, and Class 10 to indicate trees completely girdled at the crown and with most, or all, of the roots dead.

[1] Schneider, Henry, E. W. Bodine, and H. Earl Thomas. Occurrence of Armillaria root rot in Santa Clara Valley. U. S. Dept. Agr., Plant Disease Reptr. 29: 495–496. 1945.

[2] Smith, Ralph E. Diseases of fruits and nuts. California Agr. Ext. Circ. 120. 1941.

TABLE 1.—*Summary of results with plum planting at San Jose, California, for Armillaria resistance test after 17 years*

Selection	Number planted	No. surviving in 1945	No. killed by *Armillaria* in 9 years	Average degree of infection of survivors
Methley plum	40	37	0	7.6
Marianna, Tenn.	40	27	5	8.1
Do Calif.	40	32	2	7.1
Do 2624	39	35	0	6.7
Do 2623	40	29	3	7.1
Gypsie Myrobalan	40	18	4	6.2
Red Leaf Myrobalan	40	31	6	5.4
Purple Leaf Myrobalan	40	23	1	8.6
Myrobalan 29	38	30	0	7.5
Davis Myrobalan	40	27	1	8.5
Davis Myrobalan 7102	40	20	0	7.2
Goddard Myrobalan	40	7	3	8.7
Campus Myrobalan	40	30	0	7.4
Do	40	20	2	7.7
Do No. 3	37	30	4	7.0
Black Damas D	40	10	15	8.9
Do C	40	6	7	9.2
St. Julien G	40	19	1	8.4

None of the 431 survivors fell into Class 0, and only 3 trees were good enough for Class 1. One of the 3 trees was sufficiently affected by crown gall to be discarded. Attempts are being made to build up new clones from the 2 remaining trees for further tests. One of these trees is from the lot designated as Marianna 2624 which in recent years has been in favor for orchard trials against *Armillaria*. A few root cuttings survived from this single tree, but, of course, at least a year or two will be required to increase these to any considerable number of trees. Although the Red Leaf Myrobalan

TABLE 2.—*Rating of some of the more resistant trees in the San Jose plot*

Botanical name	Common name	No. planted	No. of survivors	Average rating
Castanea dentata (Marsh.) Borkh.	American chestnut	40	27	0.74
Castanea sativa Mill.	Spanish chestnut	40	32	0.86
Diospyros kaki L.	Japanese persimmon	40	13[a]	0.85
D. lotus L.	Date-plum	40	36	0.88
D. virginiana L.	American persimmon	40	36	0.13
Ficus carica L.	Fig (Kadota)	40	39	0.10
Ficus carica L.	Black mission fig	39	28	0.25
Juglans hindsii Sarg.	Northern Calif. Black walnut	40	35	0.77
Pyrus betulaefolia Bunge.	Pear	39	15	2.93
Pyrus calleryana Decne.	Pear	40	24	1.90
Pyrus communis L.	Common cultivated pear	40	25	1.04
Pyrus malus L.	Cultivated apple "French crab" seedlings	40	31	1.10
Pyrus malus L.	Seedlings of Red Delicious apple	40	29	1.04

[a] Only one known to be killed by *Armillaria*.

had comparatively good average rating, survival was poor, and this lot contained no tree better than Class 2. The Myrobalan 29 of this test was not clearly better than some of the others, though trees under this number have been rather extensively planted in orchards. It now seems likely, however, that several different types of Myrobalan have been distributed under this number.

A number of other types of fruit and nut rootstocks were uprooted and examined in 1946 (Table 2). Some of these were planted 2 years later than the plum roots referred to in Table 1. Fig was least affected of all the trees examined, and the damage to this tree was slight. The best persimmon was hardly more affected. The row of Northern California Black walnut (*Juglans hindsii*) represented in Table 2, originated from seed of an isolated tree not likely to have been exposed to pollen of the susceptible Persian walnut. This row was rated about as expected on the basis of the oft-reported resistance of this species. It is probable that this lot of walnut and the figs would have rated even better had not meadow nematode and other agencies aided penetration by *Armillaria*. However, another row of black walnut under the same name and with no indication of hybridity, displayed little, if any, greater resistance than the plum roots which the black walnut is replacing in many areas infested by *Armillaria*. Some lots of apple scored surprisingly well in view of the fact that orchard trees are frequently killed by *Armillaria*. The best apples approached the pear in rating, the latter being seldom, if ever, killed by this fungus in orchards. The French pear (*Pyrus communis*) lived up to expectations and was definitely more resistant than the quince which is often used as a rootstock for some pear varieties. A comparatively small number of cherry rootstocks was available for observation, and these were not examined in detail. It was evident, however, that the Mazzard root was distinctly more resistant than Mahaleb cherry or the sour cherry.

The good score of several of the lots in Table 2 indicates that this test, though intentionally severe, was not too severe.

The wide differences in amount of infection between individual trees in single lots indicate that only single clones propagated by root or stem cuttings, when possible, should be used in such trials in the future.—HAROLD E. THOMAS, H. EARL THOMAS, CATHERINE ROBERTS, and AL AMSTUTZ, Division of Plant Pathology, University of California, Berkeley and San Jose.

The Microscopic Detection of Bacterial Infections in Plants by Means of Oblique Illumination.—F. C. Stewart observed the oozing of bacteria from the cut surface of infected plant tissues into a surrounding film of water as early as 1897.[1] This method of detecting bacterial infections was later used by Jones, Johnson, and Reddy[2] and by numerous others. As far

[1] Stewart, F. C. A bacterial disease of sweet corn. N. Y. Agr. Exp. Sta. Bul. 130. 1897.

[2] Jones, L. R., A. G. Johnson, and C. S. Reddy. Bacterial blight of barley. Jour. Agr. Res. [U.S.] 11: 625–643. 1917.

as we can determine direct lighting by means of the bright field condenser of the microscope has always been used when the microscope has been used to make this so-called "ooze test."

We have found that the use of oblique illumination to produce a dark field brings out the oozing bacteria much more clearly than does direct lighting. With oblique illumination the mass of oozing bacteria appear as a brilliant white column against a dark background (Fig. 1, A).

No special equipment is needed for oblique illumination. A microscope lamp producing light of fairly high intensity is satisfactory as a light source. The oblique illumination is produced by removing the condenser from the microscope and moving the concave mirror over to one side (Fig. 1, B). If the microscope does not have a concave mirror which can be

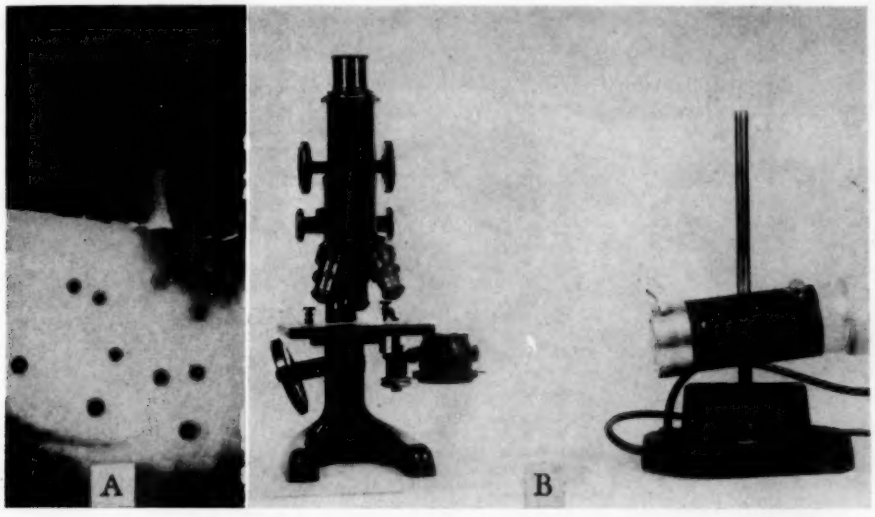

FIG. 1. A. Bright column of bacteria oozing from a necrotic spot in a cotton leaf. B. Arrangement of microscope and lamp for oblique illumination.

swung to one side the mirror may be attached to a ringstand and held in the oblique position.

The 16-mm. low power objective must be used in order to obtain a satisfactory dark field with this simple system of illumination.

In the case of bacterial leaf spots a cut through the middle of an infected spot is made with a razor blade, the cut tissue is mounted on a slide under a cover glass and a drop of clean water is added at the edge of the cover glass. With stems the cut end of a stem may be mounted in a drop of water without a cover glass or thick longitudinal sections may be mounted in water under a cover glass. The cover glass should not be touched after mounting if the bacteria are to remain near the point where they ooze from the cut end of the tissues.—T. E. RAWLINS and P. R. DESJARDINS, Division of Plant Pathology, University of California, Berkeley.

Rhizoctonia Rot of Tulips in the Pacific Northwest.[1]—A greenhouse planting of William Pitt tulips from Spokane, Washington, showed a top rot bulb infection in January, 1947. The injury was so severe that 7,000 out of 15,000 bulbs were either destroyed or injured to the extent that they did not produce marketable flowers. The infected bulbs were from two sources only, one said to be imported, the other one year from importation. Shipments of other varieties in the same greenhouse had no injury.

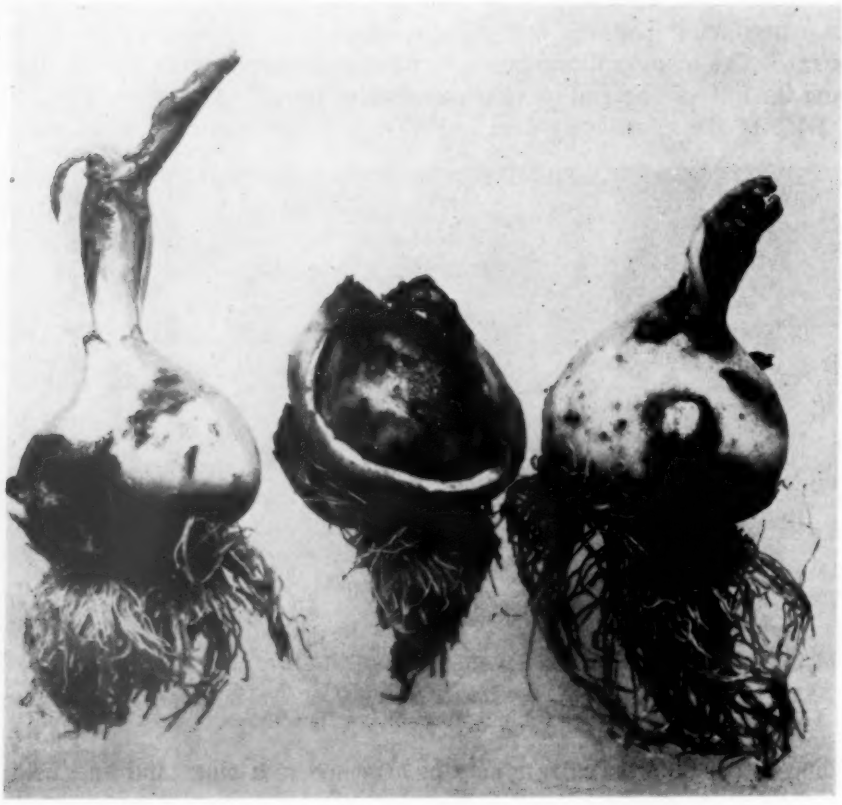

FIG. 1. Symptoms of *Rhizoctonia solani* injury on William Pitt tulips. (Photo by Sprague and MacLean.)

The symptoms somewhat resembled Botrytis fire but with certain distinct differences. The lesions on the bulbs were gray or gray-brown and somewhat dry (Fig. 1). The leaves from diseased bulbs were either killed or stunted, frequently adhered together, and bore the gray or gray-brown lesions. A white cottony mycelium grew on the necks of some of the bulbs. In severe cases the bulbs were almost rotted away. The roots of all except the most severely injured were still active, however.

Isolations were obtained from necrotic bulb parts on potato-dextrose agar, bean-pod agar, and prune agar. The mycelium developing from the

[1] Published as Scientific Paper No. 721, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington.

sections grew at only moderate speed, after a slow start. The light-brown mycelium had the characteristic right-angled branching and constrictions of *Rhizoctonia*. Hyphal strands measured from 6–8 μ in diameter (average 7 μ). The causal agent appears to be *Rhizoctonia solani* Kuehn.

In addition to the common Rhizoctonia mold, the usual species of *Penicillium* were present and also nematodes, mites, and *Pythium ultimum* Trow, none of which appeared to be the primary cause of the injury. However, the soft decay which followed the dry rot no doubt increased the speed of the bulb's decline.—NEIL ALLAN MACLEAN, State College of Washington, Pullman, Washington.

The Occurrence of South American Leaf Blight of Hevea Rubber Trees in Mexico.—Hevea rubber tree plantings in Central America north of Costa Rica and in Mexico have been considered as being free from infection by *Dothidella ulei* P. Henn., the cause of South American leaf blight of Hevea.[1] Although many plantings in these countries have been under close observation since 1941, leaf blight has not been found in any of them until recently.

On September 26, 1946, the presence of South American leaf blight was discovered by the writer and T. D. Mallery in a seedling nursery near Teapa, Tabasco, in southern Mexico. Indications were that the infection was not more than 3 months old. In an attempt to trace the origin of the disease in Mexico, a survey was started of all the Hevea plantings in the Tabasco-Chiapas rubber zone. New infections were found in most of the one-year-old seedling nurseries near Teapa. Since these nurseries had originated from disease-free seed of mature Hevea trees at El Palmar in the state of Veracruz, it was assumed that the infection had originated from some of the early Hevea plantings in Tabasco or Chiapas. A few scattered old trees near Teapa had only relatively new infections.

In the states of Tabasco and Chiapas there are 10 or more isolated plantings of Hevea rubber trees which were started about 1910 and later abandoned. Although only a few trees have survived in most of these plantings, the one at Las Palmas, in the northwestern part of the state of Chiapas, contains 18,000 trees. An inspection of the trees at Las Palmas revealed evidence of leaf-blight infection which was at least two years old. The trees had been inspected and found to be blight-free in 1941. This evidence indicated that some other planting in the zone was the original source of infection, since there had not been any known introductions of foreign Hevea material into the zone during recent years.

Inspections of the other old Hevea plantings known to exist in the Tabasco-Chiapas zone revealed the probable source of South American leaf blight of *Hevea* in Mexico. A planting of approximately 800 trees located along the Chumulá River near Chuctiepá in northeastern Chiapas, approxi-

[1] Langford, M. H. South American leaf blight of Hevea rubbertrees. U. S. Dept. Agr. Tech. Bul. 882. 1945.

mately 40 miles east to northeast of Yajalon, Chiapas, was heavily infected with leaf blight and evidence indicated that it had been infected many years. This planting had been made about 1910 with small stumped seedlings, the origin of which has not been determined. It is probable that the fungus was introduced with this original planting material. There are several possibilities as to the mode of spread of the pathogen to the other Hevea plantings in the Tabasco-Chiapas zone. The probable spread was from numerous volunteer seedlings, growing underneath the old Hevea trees at Chumulá, which were obtained by a farmer near Teapa and sold to other farmers in the region.

On October 11, 1946, initial infections of leaf blight were first observed by Julius Matz in the large seedling nurseries at the Campo Experimental de Hule at El Palmar in the state of Veracruz. Evidence points to the exchange of workers as the probable means by which the pathogen was introduced into the El Palmar plantings from the Tabasco-Chiapas zone.

A close survey of the Hevea plantings in Guatemala during November, 1946, failed to show any leaf blight in those plantings. The plantings in Honduras, El Salvador, Nicaragua, Haiti, and the Dominican Republic apparently are also blight-free.

The hitherto restricted occurrence of leaf blight in Mexico greatly simplified the initiation of the Hevea planting program in that country since 1941. Its present widespread distribution, while somewhat unexpected, should not interfere with the progress of the program, since there is a good supply of resistant Hevea clones available in Mexico for use in top budding the high-yielding but susceptible clones being planted.—W. J. MARTIN, formerly in Division of Rubber Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Urocystis agropyri on Phleum pratense.[1]—Stripe smut of timothy in the northeastern United States is commonly caused by *Ustilago striiformis* (West.) Niessl. Formation of linear sori that eventually rupture and lead to shredding of leaves is a characteristic symptom of the disease. In the fall of 1944, Kreitlow and Cassell[2] collected a smut on timothy near Brandon, Vermont, that was indistinguishable macroscopically from *U. striiformis*, but when spores of the smut were examined with a microscope they were found to consist of spore balls resembling those of *Urocystis agropyri* (Preuss) Schroet. No previous report of this fungus on *Phleum pratense* L. was found.

The identical symptoms of the two diseases suggested that perhaps some specimens of timothy identified macroscopically as infected by *Ustilago*

[1] Contribution No. 85 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture, State College, Pa., in cooperation with the Northeastern States.

[2] Kreitlow, K. W. and Robert C. Cassell. *Phleum pratense* L., a new natural host for *Urocystis agropyri*. U. S. Dept. Agr. Pl. Dis. Reptr. 29: 365. 1945.

striiformis might in reality be attacked by *Urocystis agropyri*. Travel restrictions during the war prevented further collections, but in June, 1946, and in 1947, smutted timothy was collected in several northeastern states. The field where *Urocystis* was first obtained was revisited in 1946, but the timothy was gone and no smutted plants were located. Of the specimens collected in 1946, all but one were identified as *U. striiformis*. The exception was a collection of *Urocystis agropyri* obtained near Walpole, New Hampshire. No specimens of *Urocystis* were obtained among the samples of smutted timothy collected in 1947.

A highway distance of approximately 75 miles separated the two samples of *Urocystis agropyri* collected on timothy. In this connection, a collection of smutted timothy made by Dr. Charles Drechsler in 1917 near Warrensburg, Missouri, should be mentioned. The causal organism was identified as *Urocystis agropyri* and a specimen was deposited in the Mycological Collections of the Division of Mycology and Disease Survey. No report of the collection was published. Drechsler's specimen was examined and the spores found comparable to those obtained in Vermont and New Hampshire. This suggests the smut is not localized or restricted to the northeast.

To date, 26 samples of smutted timothy have been collected in the following states: Maine, New Hampshire, Vermont, New York, Connecticut, Massachusetts, New Jersey, West Virginia, and Pennyslvania. Microscopic examination, however, showed that all but two of the specimens were infected by *Ustilago striiformis*.

Spore balls of the smut on timothy resembled *Urocystis agropyri*, from other species of grasses, in general characteristics such as size, shape, and color. There was, however, a predominence of single-spored spore balls.[3] The limited herbarium material available prevented extensive experimental work with the smut. However, tests were conducted to induce germination of spores by exposing them to a temperature of 30° C. in a moist chamber for 10 to 14 days or by soaking spore balls in different dilutions of benzaldehyde. None of these measures yielded germinating spores.

The few specimens of *Urocystis* collected on timothy to date suggest that this smut occurs infrequently on timothy. However, unless microscopic examinations are made, the similarity in symptoms of the two smuts may result in attributing some cases of infection by *Urocystis agropyri* to *Ustilago striiformis*.—K. W. KREITLOW, U. S. Regional Pasture Research Laboratory, State College, Pennsylvania.

[3] In a personal communication, Dr. George W. Fischer verified the identification of the smut and pointed out the predominence of single-spored spore balls.

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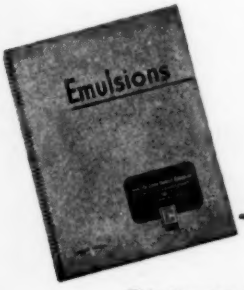

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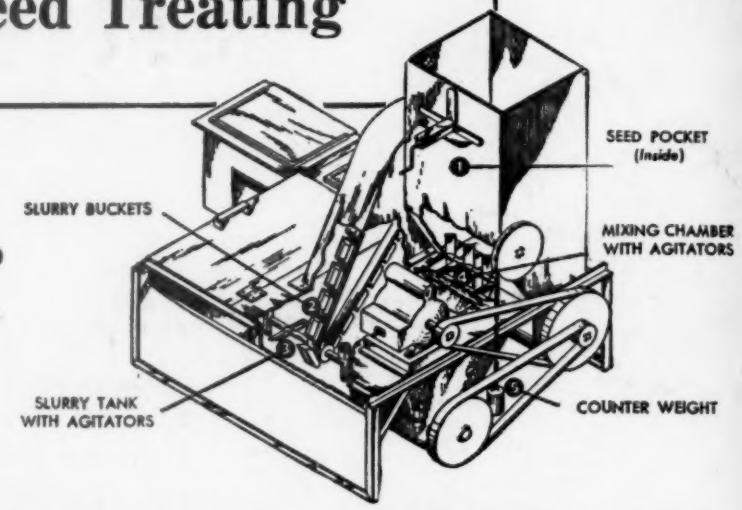

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